STUDY ON THE PREVALENCE OF SEXUALLY TRANSMITTED INFECTIONS WITH SPECIAL PREFERENCE TO GENITAL HERPES INFECTION IN THE HIGH RISK GROUPS ATTENDING STD CLINIC IN A TERTIARY CARE HOSPITAL

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THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

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DECLARATION

I solemnly declare that this dissertation "***STUDY ON THE PREVALENCE OF SEXUALLY TRANSMITTED INFECTIONS WITH SPECIAL PREFERENCE TO GENITAL HERPES INFECTION AND OTHER MICROBIAL INFECTIONS IN THE HIGH RISK GROUPS ATTENDING STD CLINIC IN A TERTIARY CARE HOSPITAL***" is the bonafide work done by me at the Department of Microbiology, Govt. Stanley Medical College and Hospital, Chennai, under the guidance and supervision of **Prof. Dr. R. SELVI, M.D.,** Professor of Microbiology, Govt. Stanley Medical College, Chennai-600 001.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfilment of the University regulations for the award of degree of M.D. Branch IV Microbiology examinations to be held in April 2015.

Place: Chennai.

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CERTIFICATE

This is to certify that this dissertation entitled "***STUDY ON THE PREVALENCE OF SEXUALLY TRANSMITTED INFECTIONS WITH SPECIAL PREFERENCE TO GENITAL HERPES INFECTION AND OTHER MICROBIAL INFECTIONS IN THE HIGH RISK GROUPS ATTENDING STD CLINIC IN A TERTIARY CARE HOSPITAL***" is the bonafide original work done by **Dr.S.ARULSELVAN,** Post graduate in Microbiology, under my overall supervision and guidance in the department of Microbiology, Stanley Medical College, Chennai, in partial fulfilment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D Degree in Microbiology (Branch IV).**

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CONTENTS

ABSTRACT

TITLE:STUDY ON THE PREVALENCE OF SEXUALLY TRANSMITTED INFECTIONS WITH SPECIAL PREFERENCE TO GENITAL HERPES INFECTION IN THE HIGH RISK GROUPS ATTENDING STD CLINIC IN A TERTIARY CARE HOSPITAL.

INTRODUCTION:

High risk group of people attending the STD clinic including Female sex workers, males having sex with males and transgenders harbors the high risk of encountering the sexually transmitted infectionsmaking them more vulnerable in acquiring sexually transmitted infections and also spreading it to the community.

AIMS AND OBJECTIVES:

- To determine the prevalence of sexually transmitted viral infections like Human immunodeficiency virus, Herpes simplex virus 2, Human papilloma virus 16 and 18, hepatitis B and C virus and Gonorrhea, Bacterial Vaginosis, syphilis, vaginal candidiasis and Trichomoniasis among asymptomatic high risk groups of males who have sex with males, Transgenders and female sex workers attending STD clinic in a tertiary care hospital.

- Comparison of seroprevalence of herpes simplex virus 2 and genital viral shedding among the high risk groups
- To determine the prevalence of high risk Human Papilloma virus 16 & 18 viral shedding among the high risk groups

MATERIALS AND METHODS:

Specimen collection and processing:

Samples from Female sex workers, Males having sex with males, transgender including Endocervical swabs, High vaginal swab, rectal swabs, Urethral swabs, pharyngeal swab and bloodare collected and processed by standard microbiological methods. Pharyngeal and rectal specimens were obtained using cotton swabs. Rectal specimens were obtained either by blind anal swabbing or, via anoscopy.Cervical specimens taken with Dacron swabs are preserved with 70% ethanol solution for molecular analysis. Serological tests done for ELISA HIV, HSV2 IgM and IgG ELISA. Serological testing done for Syphilis with RPR test and Confirmation with Treponema pallidum hemagglutination test (TPHA).Rapid card test done for hepatitis B and Hepatitis C.

PCR done from the anogenital swabs for HSV2, HPV 16 and 18

RESULTS:

Among the high risk groups taken for study the female sex workers (57%) were the commonest and most of them are unmarried 106(53%) with

predominant mode of sexual contact was heterosexual mode (57%) and 41% had unprotected sexual act (without condoms).

Out of 200 asymptomatic cases *Neisseria gonorrhoeae (1), Trichomonas vaginalis (1), Candida albicans 9 and non albicans 9, Gardernerella vaginalis (4)* were isolated. HIV reactivity (8), HbsAg (1) and RPR reactivity (7). IgM, IgG antibodies for *HSV 2* is (16) and IgG (67) respectively and PCR for HSV2 is 20.Speciation of the candida species showed candida Human *Papilloma Virus* 18 *E7* and 18 *E7* in 4 with one positive for both HPV 16 and 18 co infection.

CONCLUSION:

Out of 20 PCR positives the IgM was positive in only 16.There was genital viral shedding even in the absence of IgM antibodies. So molecular methods are necessary to know about the genital viral shedding.

Continuous efforts are needed to encourage individuals to adopt safer sexual. Prophylactic strategies regarding antiviral therapy for HSV2 infected individuals and vaccination for HPV to be considered in the high risk groups.

Key words-Sexually transmitted infections, High risk groups, Genital Herpes,

INTRODUCTION

Historically sexually transmitted diseases are as old as mankind, scholar's claim that the references of venereal diseases has been in biblical versions. The incidence of four sexually transmitted infections like Trichomoniasis, Gonorrhoea, Chlamydiasis and syphilis is estimated to be 489.9 million worldwide[1].

Even with the increased awareness and availability of effective treatment Sexually Transmitted Infections are still at a large, causing significant morbidity and mortality worldwide. They remain to be the important cause of acute illnesses, impotence, infertility, long term disability and death globally.

There are over more than 20 causes of sexually transmitted infections including bacterial, viral and parasitic agents. The prevalence of bacterial causes of STIs like syphilis, Gonorrhoea, Chlamydiasis, Bacterial vaginosis and the parasitic Trichomoniasis is more in the developing countries compared to industrialised nations where there is STIs of viral infections is more but now the trend has changed due to the spread of HIV throughout the world making the incidence of viral infections like Herpes simplex virus 2, Human papilloma virus, Hepatitis B and Hepatitis C and Cytomegalovirus predominate globally. This increased incidence of viral infections can also be due to the increased awareness of people seeking healthcare facilities, increased use of broad spectrum antibiotics and the effectiveness of syndromic approach of treatment[2].

Sexually transmitted infections increase the risk of acquiring and transmission of HIV so the presence of untreated STIs (ulcerative or non-ulcerative) increases the risk of acquiring and transmitting the HIV by a factor of ten[3]. In developing countries STIs and its complications are the one of the top five disease categories for which adults seeks healthcare facilities.

Hepatitis B virus and Hepatitis C virus infection is an important health problem in the developing countries like India. Carrier rates of HBV and HCV in India was 3% and 1-1.5% respectively and about 2 lakh people die annually because of HBV or HCV alone[6]. About 240 million people are affected with chronic HBV infection[5] worldwide. Since HBV and HIV shares the same route of transmission the prevalence of HBV in the high risk population attending the STD clinic is more compared to the general population[4].

Various population-based studies conducted in developed and developing countries have reported Genital Herpes Simplex Virus 2 Prevalence in the range of 5.6-42.2%[7].Genital herpes virus infection is an important risk factor for the infection of HIV and also for syphilis. The prevalence of HSV2 varies according to the population taken for the study. For instance the prevalence of HSV2 in the high risk group attending the STD clinic is about 70% and the prevalence in the antenatal mothers is about 11.3% in developing countries[7].

Mostly genital herpes infection remains asymptomatic / subclinical and undiagnosed. As per WHO treatment regime 25 cases of first/primary episode

of genital herpes infection is missed clinically. Genital herpes is mainly caused by Herpes simplex virus 2 and also rarely by herpes simplex virus 1. Both the symptomatic and asymptomatic patients will be having the intermittent viral shedding from their genital tracts during which they will be transmitting the disease[9] and in the asymptomatic patients genital shedding of virus happens in 10% of days during which the patient don't have any signs or symptoms[13] . The prevalence of genital viral shedding during pregnancy ranges from 0.5 to 0.6%[14] by which the child can acquire the infection. Neonatal infection is mostly symptomatic and lethal which in the absence of intervention will be around 80%[14.] The High risk patients with Genital herpes will be potentially spreading the genital herpes to their contacts and spouses making them vulnerable to other infections like HIV and syphilis.

So it is important to control the viral shedding thereby preventing the disease spread. With antiviral therapy the genital viral shedding can be reduced[9].So it is necessary to adopt a serological/Molecular methods to diagnose the asymptomatic high risk cases and for the intervention measures like prophylactic antiviral treatment and protective (barrier) measures.

Human papillomavirus (HPV) is the most common sexually transmitted viral infection and studies have estimated that globally 50-80% of sexually active men and women are infected with the virus at least once during their lifetimes. Globally about 291 million women are HPV DNA carriers. About

7.9% of women in the general population are estimated to harbor cervical HPV infection at a given time.

India has a population of 432.20million women in the age group of 15 years and above who are at risk of developing cervical cancer. Currently it has been estimated that every year 1,22,844 women are diagnosed with cervical cancer and 67477 die from the disease. Cervical cancer ranks as the 2nd most frequent cancer among women in India and84.1% of invasive cervical cancers are attributed to HPVs 16 or 18[22].The HPV virus is associated not only with cervical cancer but also with anal, vaginal, penile and oral cancers. Besides HPV, genital tract infections from other organisms like *Trichomonas vaginalis* (TV), *Chlamydia trachomatis and Herpes simplex virus* (HSV) type 2 have also been implicated in cervical cancers in women. Human Immunodeficiency virus (HIV) positive women have significantly higher prevalence of genital squamous intraepithelial lesions and of multifocal HPV related diseases[29].

Studies have indicated that a high number of lifetime partners may lead to a higher transmission of HPV leading to higher cervical cancer rates. In female sex workers (FSWs), the risk of HPV infection and cervical cancer is especially high. In addition, HPV can be transmitted from FSWs to the general population through clients thereby increasing the prevalence of the virus[28].

The female sex workers (FSW), the men having sex with men (MSM) and transgender (TG) are at high risk of contracting various sexually transmitted

infections (STI) due to their high risk sexual behaviors. Co-existence of HPV and other sexually transmitted infections can increase their predisposition to different ano-genital cancers[29].

Neisseria gonorrhoeae infection one of the commonest STIs which is estimated to be about 106.1 million new cases worldwide. The prevalence of N.gonorrhoeae in the adult males(age15-49yrs) is 1% in south and southeast Asia[3].N.gonorrhoeae apart from being a risk factor for acquiring HIV it is also the causative factor for PID, infertility, ectopic pregnancy and chronic pelvic pain. There has been increased incidence in the treatment resistant strains of N. gonorrhoeae making the management of gonococcal infection a challenge. There has been wide difference in the resistance pattern throughout the India with penicillin resistance being 20% to 79%, ciprofloxacin 10.6% to 100% and tetracycline resistance 0% to 45.6%[8]. So studying about the treatment resistant strains will help in updating the local treatment guidelines.

Syphilis caused by Treponema pallidum is one of the oldest known STI in the world with the worldwide prevalence of 14.3 million.Eventhough the prevalence is declining currently there has been increased incidence among the high risk population especially men having sex with men co-infected with HIV[10].This change in trend may be due to the spread of HIV and change in the sexual behaviour pattern. Screening and diagnosing syphilis with RPR and

TPHA in the high risk groups will help in curtailing the spread of syphilis and HIV among these high risk groups and also to the general population.

Vaginal discharge in the reproductive age group of women is most commonly is caused by Trichomoniasis, bacterial vaginosis and vaginal candidiasis of which 276.4 million total number of new cases of Trichomonas vaginalis is seen globally[1] and out of which 28.7 million cases were in southeast Asian region with the male and female prevalence is about 5.6% and 0.6% respectively. Trichomoniasis is the commonest non-viral STI and it had been suggested as one of the important causes of non gonococcal urethritis and also it amplifies the risk of HIV transmission[11].

Bacterial vaginosis is one of the commonest causes of vaginal discharge in the women of child bearing age group and associated with the low birth weight and many studies throughout the world had shown the association of bacterial vaginosis with other STIs like Genital herpes infection, Trichomoniasis ,Gonococcal infection and HIV [15].

Vulvovaginal candidiasis which is said to be affecting 75% of women in their lifetime can be asymptomatic but also causes significant morbidity including vaginal discharge, dysuria, itching and dyspareunia and also 50% of women suffer from recurrent episodes[16.]

Sexually transmitted infections causes significant morbidity to the high risk patients and also making them more vulnerable in acquiring sexually

transmitted infections like HIV and also spreading it to the community .Asymptomatic Genital herpes infection plays a significant role in spreading the disease and also increasing the vulnerability of acquiring the other sexually transmitted infections So the purpose of this study is to evaluate the prevalence of sexually transmitted infections so that early diagnosis and treatment would cure the disease as well preventing them from transmitting the sexually transmitted infections.

AIMS & OBJECTIVES

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- To determine the prevalence of sexually transmitted viral infections like Human immunodeficiency virus, Herpes simplex virus 2, Human papilloma virus 16 and 18, hepatitis B and C virus and Gonorrhea, Bacterial Vaginosis, syphilis,vaginal candidiasis and Trichomoniasis among asymptomatic high risk groups of males who have sex with males, Transgenders and female sex workers attending STD clinic in a tertiary care hospital.
- Comparison of seroprevalence of herpes simplex virus 2 and genital viral shedding among the high risk groups
- To determine the prevalence of high risk Human Papilloma virus 16 & 18 viral shedding among the high risk groups

REVIEW OF LITERATURE

Sexuality is a fundamental aspect of human life, carrying the potential to create new life and fulfilling both personal and social needs. Sexual health as per WHO is defined as" Sexual health is a state of physical, mental and social well-being in relation to sexuality. It requires a positive and respectful approach to sexuality and sexual relationships, as well as the possibility of having pleasurable and safe sexual experiences, free of coercion, discrimination and violence"[19] .To attain this sexual health we need to have proper understanding of multi-level determinants of STIs and to refocus about the prevention and control strategies of sexually transmitted infections.

The term sexually transmitted infections had undergone various changes over the century initially known as 'Venereal disease' and later on as 'sexually transmitted diseases' finally as 'sexually transmitted infection' which is a broader terminology including those who have infection and spreading it without having the disease.

Sexually transmitted infections are the infections which are spread by person to person sexual route including vaginal, anal, oral and skin to skin contact and some sexually transmitted infections which are also spread through non sexual routes like blood transfusion, IV drug abuse, congenital, child birth and breast feeding.

There are more than 30 causes of STI which includes bacterial, viral, fungal and parasitic organisms. Of these organisms 8 organisms are linked with

the greater incidence of illnesses. Among these 8 infections four of the currently curable diseases are syphilis, Trichomoniasis, Chlamydiasis and gonorrhoea and other four are viral infections including HIV, Hepatitis virus, Herpes virus and HPV which are even though incurable but can be mitigated or modulated with treatment [17] .

Among the STIs the infections like HIV, syphilis, gonorrhoea and hepatitis B is seen more in the high risk populations like commercial sex workers, men having sex with men (MSM) and the drug abusers whereas other infections like genital herpes, human papilloma virus infection and chlamydial infection can easily spread among the low risk population[18].

STIs causing genital ulcers are more important as the genital ulcers increases the risk of acquiring the HIV infection and also shedding the virus and thereby increasing the spread of the disease. Causes of genital ulcers are syphilis, herpes, chancroid, Lymphogranuloma venereum and Donovanosis of which the syphilis, chancroid and herpes are important and commonest causes. With increased efforts to control chancroid and syphilis has led Genital herpes to be commonest cause in the developing countries[17].

VIRAL CAUSES OF STI

GENITAL HERPES INFECTION

'Herpes' a Greek word meaning 'to crawl or creep' referring to the spreading nature of the herpetic lesions. The references of lesions similar to genital herpes have been seen in Sumerian tablet from the third millennium BC and in the Ebers Papyrus (circa 1500 BC).

Genital herpes was more recognised after the advances in science of virology in the middle 20th century and also the first reliable antiviral agent Acyclovir was developed to treat Genital and oral herpes in the early 1980s[19].

Herpes simplex virus is classified as alpha herpes virus under the herpes virus family .these alpha viruses are fast growing, cytolytic and have latent infection in neurons of the host.

Genital herpes is predominantly caused by human herpes virus 2 and also by human herpes virus 1 it affects the mucosal surfaces and damaged cutaneous sites .HSV regardless of the type first enters the sensory nerves during the primary infection and then remains dormant in the neuronal cell body and causes recurrent infection and also asymptomatic shedding of virus from the genital region.

VIRION STRUCTURE

The herpes simplex virus is a spherical particle with the diameter of 186nm and contains four structures 1. Core containing the viral DNA 2.icasohedral capsid surrounding the core with 162 capsomeres3. Unstructured proteinacous layer called tegument surrounding the capsid 4. Outer lipid bilayer with spikes in its surface.

The genome of herpes virus DNA is linear, double stranded DNA molecule(molecular weight $100x10^6$ units)that encodes more than 90 transcription units with 84 identified proteins and the genomic subtype of HSV1 and HSV2 are similar .the overall genomic sequence homology between the two types is around 50% and the protein sequence homology is around 80%.many type specific regions unique to HSV1 and HSV2 exists in the genome only based on these type specific regions serological assays distinguish between the HSV1 and HSV2[18].

REPLICATION CYCLE

Viral replication cycle has both cytoplasmic and nuclear phases. Once the virus fusion and entry happens in the host cell the nucleocapsid enters the cytoplasm and many viral proteins are released .These viral proteins shutdowns the host cell protein synthesis and turns on the transcription of early viral replication α genes and the synthesis of β polypeptide which are the regulatory

proteins and enzymes needed for DNA replication. The γ group of genes constitutes for the most of the structural proteins of virus.

Viral DNA is transcribed by RNA polymerase II.Herpes simplex viruses DNA encodes for large number of enzymes involved in the DNA synthesis which is different in compared with the other DNA viruses and these enzymes are good targets for the antiviral drugs. Viral DNA synthesised is packaged into nucleocapsid and then these nucleocapsids are then transported to the cell surface by vesicular movement. The length of the replication cycle is about 18 hours.MicroRNAs which are single stranded (~22 nucleotides) plays important role in entry in to and exit of the virus from the latent phase of virus life cycle.

PATHOGENESIS

Genital Herpes simplex infection will be transmitted from the virus excreting infected person to the susceptible contact. Herpes simplex virus causes necrosis of the infected cell along with the inflammatory response. The pathologic changes due to the viral infection will be ballooning of infected cell, intranuclear Cowdry type A inclusion body, margination of chromatin and multinucleate giant cell formation.

CLINICAL FEATURES

Genital herpes caused by both HSV1 and HSV2 viruses. The primary genital herpes infection occurring in the absence of antibodies can be severe

lasting up to about 3 weeks. The genital herpes infection is characterised by vesiculoulcerative lesion of the penis in male and vesiculoulcerative lesion of the cervix, vagina, vulva and perineum in females. These painful lesions will be associated with fever, malaise, dysuria and inguinal lymphadenopathy. The complications will be extra genital lesions in about 20% of cases and aseptic meningitis in about 10% of cases. Viral excretion may be persisting for about 3 weeks[20].as there is antigenic cross reactivity exists between HSV1 and HSV2, the prior infection with HSV1 in a patient may offer some protection during the subsequent HSV2 infection making the genital infection less severe.

LATENT INFECTION

One of the interesting ability of the herpes virus biology is that's its ability to extend a lifelong latent infection in human host. After the spread from the tissue, primary site of infection the virus enters the neuronal axons the virus travels in a retrograde manner to reach the nucleus of the nerve cell body where the lytic gene expression is suppressed and the latent associated transcript (LAT) is expressed.

If the virus reactivates then the infectious virus is travels in the ante grade manner to reach the cells at or near the initial site of infection. This reactivated herpes infection can be asymptomatic or the lesions can vary from small punctate lesions not visible to naked eye to severe debilitating lesion depending upon the immune status of the individual. The recurrence of genital herpes

infection is common and tend to be mild .Few vesicles appear and tend to heal within 10 days. Sometimes the asymptomatic anogenital viral shedding may be very minimal that it lasts only for 24 hours.

The reactivation of latent viral can be due to either local causes physical injury to the initial site of infection or can be due to the systemic causes like fever, exposure to UV light and physical, emotional stress.

The frequency of recurrences in men is about 2.7 per 100 patient days and whereas for females it is 1.9 per 100 patient days. Overall the frequency of recurrences is about 60% and approximately about 90% of HSV2 infected patients will have one or more recurrences per year, 38% may be having six recurrences and 20% having 10 recurrences[14].

Viral shedding in the commercial sex workers with the age group of 20 to 29 is about 12% whereas in the older age groups of commercial sex workers the viral excretion is about 6% only. The HSV2 viral shedding among the middle and upper socioeconomic women is 0.3 to 0.2% respectively which is much lesser compared to the women attending the STD clinic[14].

LABORATORY DIAGNOSIS

CYTOPATHOLOGY

The skin scrapping's obtained from the base of the vesicle stained with Giemsa stain will show the multinucleate giant cells which is indicative of herpes virus (HSV1, HSV2 and Varicella zoster) infection.

VIRAL ISOLATION

Virus can be isolated from CSF, throat, urine, stool, nasopharynx and conjunctiva. Viral isolation is the definite diagnostic method.it is easy to cultivate and the cytopathic effects appear by 2-3 days.it is then identified by NT test or immunofluorescence staining with specific antiserum. Typing is done by monoclonal antibody or restriction endonuclease method.

POLYMERASE CHAIN REACTION

It is the most specific and sensitive method .By using primers from an HSV DNA sequence that was common to both HSV types 1 and 2 (either the gB (viral envelope glycoprotein)or DNA polymerase genes) we can identify the causes of Genital Herpes infection irrespective of its type.

SEROLOGY

Antibodies start to appear 4-7 days after the infection and will reach peak 2-4 weeks. Diagnostic value of serological assay is limited because of sharing of multiple antigens between HSV1 and HSV2. Only the type specific HSV antibodies serological tests will be of diagnostic value.

HUMAN PAPILLOMA VIRUS INFECTION

It is the most common viral infection of the reproductive tract and worldwide about 660 million people are having HPV genital infections, an estimated 6.2 million new infections are occurring annually in the United States.

The peak incidence of HPV infections occurs in adolescents and young adults under 25 years of age[20].

The Papillomaviridae family, a very large virus family is divided into 16 genera, of which five contain members that infect humans (*Alpha*-, *Beta*-, *Gamma*-, *Mupa*-, and *Nupapapillomavirus*). Using the molecular criteria more than 100 distinct human papillomavirus (HPV) types have been recovered.

The Human Papilloma Virus of greatest medical importance is those which are associated with genital and mucosal cancers and they are members of the alpha genus. Most alpha Papilloma Virus infect the external genitals, genital and non-genital mucosal surfaces are collectively called as genital-mucosa types. The high risk types that are associated with cervical cancer are species 5, 6, 7, 9, 11, 16 and 18. HPV-16, the type found most frequently in cervical cancer, is a member of species 9, whereas the next most common cancer-associated type, HPV-18, is a member of species 7. HPV6, which causes most cutaneous genital warts, is a species 10 member.

STRUCTURE:

It is an epitheliotropic, non-enveloped, double stranded DNA virus with the diameter of about 55nm having genome of about 8kbp which is surrounded 72 capsomers having nucleocapsid.

REPLICATION:

The papillomaviruses are highly species-specific and they have a specific predilection for squamous epithelial cells. The Papillomaviruses infection can be divided into early and late stages. The virus must will infect the basal cell to establish a persistent lesion and the late gene expression, capsid proteins synthesis, vegetative viral DNA synthesis, and assembly of virions occur only in terminally differentiating squamous epithelial cells.

PATHOGENESIS

Viral particles are released from the surface of papillomatous lesions will be transmitted by close contact. HPV genital infections are sexually transmitted and the most common sexually transmitted disease in the United States.

Cervical cancer is the second most frequent cancer in women worldwide (about 500,000 new cases annually) and is a major cause of cancer deaths in developing countries. Cervical cancer develops slowly taking years to decades[20]. Even though multiple factors are involved in progression to malignancy the persistent infection with a high-risk HPV is a important component to the process of malignancy.

CLINICAL FEATURES

HPVs are accepted as the cause of anogenital cancers. Over 99% of cervical cancer cases and over 80% of anal cancer cases are linked to genital

infections with HPVs. Although many different HPV types cause genital infections, HPV-16 or HPV-18 is found most frequently in cervical carcinomas, though some cancers contain DNA from other types, such as HPV type 31. Epidemiologic studies indicate that HPV-16 and HPV-18 are responsible for more than 70% of all cervical cancers, with type 16 being most common.

Condylomas are usually exophytic lesions that are frequently multiple. They can arise anywhere on the external genitalia and can be found simultaneously in multiple sites. In men, they occur most commonly on the penis and anus, and in women on the perineum and anus. The anus can develop multiple lesions that coalesce to surround the anal canal. Most condylomas are usually self-limited, regressing spontaneously or after local treatment, but some lesions can persist for years.

As with other HPV infections, genital warts, when they arise in patients with impaired cellular immunity, can be extremely refractory to treatment. They can also increase in size and number during pregnancy, and regress following delivery. This sequence of events may also be a reflection of the immune suppression associated with pregnancy.

About 90% of genital warts are caused by HPV-6 or HPV-11, which are closely related to each other, with HPV-6 predominating. Other HPV types, including HPV-16, may also be found in these lesions. Most of these lesions

contain HPV-16, but the rate of transition to frank malignancy appears to be much lower for the external genitalia than for the cervix.

Anal cancer is associated with high-risk HPV infection. Immunocompromised patients are especially at risk, as are men who have sex with men. Oropharyngeal cancers, a subset of head and neck squamous cell carcinomas, are also linked to HPV infections, especially by type 16.

The role of men as carriers of HPV as well as vectors for transmission of infections is well documented; however, most penile HPV infections in men are subclinical and do not result in HPV-associated disease.

Anogenital warts are usually (90%) caused by low-risk HPV types 6 and 11. HPV-6 and HPV-11 cause benign genital condylomas. The infection is acquired during passage through the birth canal of a mother with genital warts.

There is a high prevalence of HPV DNA in normal skin from healthy adults. It appears that these asymptomatic HPV infections are acquired early in infancy. A great multiplicity of HPV types are detected in normal skin. Transmission is thought to occur from those in close contact with the child, with a high concordance (about 60%) between types detected in infants and their mothers.

Immunosuppressed patients experience an increased incidence of warts and cancer of the cervix. All HPV-associated cancers occur more frequently in persons with HIV/AIDS.

PREVENTION & CONTROL

Vaccines against HPV are expected to be a cost-effective way to reduce anogenital HPV infections, the incidence of cervical cancer, and the HPV-associated health care burden. A quadrivalent HPV vaccine was approved in the United States in 2006 and a bivalent vaccine in 2007. Both are noninfectious recombinant vaccines containing virus-like particles composed of HPV L1 proteins. The quadrivalent vaccine contains particles derived from HPV types 6, 11, 16, and 18, whereas the bivalent vaccine contains particles from types 16 and 18. Both vaccines are effective at preventing persistent infections by the targeted HPV types and the development of HPV-related genital precancerous lesions. They are not effective against established HPV disease. Adolescent and young adult females make up the initial target population for vaccination. It is not known how long vaccine-induced immunity lasts, but it appears to extend for at least 5 years[14].

HPV vaccines are not recommended for pregnant females.

HEPATITIS B

Hepatitis B virus infection is distributed worldwide. There are more than 250 million carriers of Hepatitis B Worldwide and 1 million deaths a year are due to to HBV-related liver disease and hepatocellular carcinoma.

Depending on the age at time of infection and the transmission routes, response of the infection vary. Mostly individuals infected as infants develops chronic infections as they reach adulthood they are subject to liver disease and are at high risk of developing hepatocellular carcinoma. There is no any seasonal trend for HBV infection and no high predilection for any age group

High-risk groups

Highly promiscuous persons, parenteral drug abusers, health care personnel, multiply transfused patients, organ transplant patients, haemodialysis patients and staff, and new-born infants born to mothers with hepatitis B.It also spreads by improperly sterilized syringes, needles, or scalpels and even by tattooing or ear piercing. After the institution of mandatory screening of blood donors for HBsAg, the number of cases of transfusion-associated hepatitis has been reduced dramatically.

Routes of transmission

More than 1 billion virions is present in one millilitre of blood from an HBeAg-positive carrier and as these viruses are resistant to drying, it should be

considered that all bodily fluids from HBV-infected patients are infectious .HBsAg can be detected in body fluids like saliva, nasopharyngeal washings, semen, menstrual fluid, and vaginal secretions as well as in blood

Transmission from carriers to close contacts by the oral route or by sexual or other intimate exposure occurs. There is strong evidence of transmission from persons with subclinical cases and carriers of HBsAg to homosexual and heterosexual long-term partners. Transmission by the faecal-oral route has not been documented. Subclinical infections are common, and these unrecognized infections represent the principal hazard to hospital personnel.

Health care personnel (medical and dental surgeons, pathologists, other physicians, nurses, laboratory technicians, and blood bank personnel) have a higher incidence of hepatitis and prevalence of detectable HBsAg or anti-HBs than those who have no occupational exposure to patients or blood products. The risk that these apparently healthy HBsAg carriers (especially medical and dental surgeons) represent to the patients under their care remains to be determined but is probably small.

Hepatitis B infections are common among patients and staff of haemodialysis units. As many as 50% of the renal dialysis patients who contract hepatitis B may become chronic carriers of HBsAg compared with 2% of the staff group, emphasizing differences in the host immune response. Family contacts are also at increased risk.

Incubation period

The incubation period of hepatitis B is 50–180 days, with a mean between 60 and 90 days. It appears to vary with the dose of HBV administered and the route of administration, being prolonged in patients who receive a low dose of virus or who are infected by a nonpercutaneous route.

Structure

HBV is classified as a hepadnavirus.

Virion:

It is About 42 nm in diameter overall (nucleocapsids, 18 nm) .Electron microscopy of HBsAg-positive serum reveals three morphologic forms the most numerous are spherical particles measuring 22 nm in diameter .These small particles are made up exclusively of HBsAg—as are tubular or filamentous forms, which have the same diameter but may be over 200 nm long—and result from overproduction of HBsAg. Larger, 42-nm spherical virions (Dane particles) are less frequently observed.

Genome:

One molecule of double-stranded DNA, circular, 3.2 kbp. In virion, negative DNA strand is full length and positive DNA strand is partially complete. The gap must be completed at beginning of replication cycle.

There are four open reading frames that encode seven polypeptides. These include structural proteins of the virion surface and core, a small transcriptional Trans activator (X), and a large polymerase (P) protein that includes DNA polymerase, reverse transcriptase, and RNase H activities. The S gene has three in-frame initiation codons and encodes the major HBsAg, as well as polypeptides containing in addition pre-S2 or pre-S1 and pre-S2 sequences. The C gene has two in-frame initiation codons and encodes HBcAg plus the HBe protein, which is processed to produce soluble HBeAg.

Replication of Hepatitis B Virus

The infectious virion attaches to cells and becomes uncoated. In the nucleus, the partially double-stranded viral genome is converted to covalently close circular double-stranded DNA (cccDNA). The cccDNA serves as template for all viral transcripts, including a 3.5-kb pregenome RNA. The pregenome RNA becomes encapsulated with newly synthesized HBcAg. Within the cores, the viral polymerase synthesizes by reverse transcription a negative-strand DNA copy. The polymerase starts to synthesize the positive DNA strand, but the process is not completed. Cores bud from the pre-Golgi membranes, acquiring HBsAg-containing envelopes, and may exit the cell. Alternatively, cores may be reimported into the nucleus and initiate another round of replication in the same cell.

INCUBATION PERIOD

50–180 days

CLINICAL FINDINGS

In viral hepatitis, onset of jaundice is often preceded by gastrointestinal symptoms such as nausea, vomiting, anorexia, and mild fever. Jaundice may appear within a few days of the prodromal period, but anicteric hepatitis is more common.

Extra hepatic manifestations of viral hepatitis (primarily type B) which are proposed to be due to circulating immune complexes includes transient serum sickness-like prodrome consisting of fever, skin rash, and polyarthritis, necrotizing vasculitis (polyarteritisnodosa) and glomerulonephritis. Uncomplicated viral hepatitis rarely continues for more than 10 weeks without improvement. Relapses occur in 5–20% of cases and are manifested by abnormalities in liver function with or without the recurrence of clinical symptoms.

Sometimes acute viral hepatitis can cause more extensive damage preventing organised liver cell regeneration. Only 1–2% of jaundiced patients with hepatitis B have such fulminant or massive hepatocellular necrosis. It is ten times more common in those coinfected with HDV than in the absence of HDV.

Both HBV and HCV have important roles in the development of hepatocellular carcinoma that may appear many (15–60) years after establishment of chronic infection.

LABORATORY FEATURES

HBsAg is generally evident 2–6 weeks in advance of clinical and biochemical evidence of hepatitis and continues throughout the clinical course of the disease but characteristically disappears by the sixth month after exposure. In the initial stages of disease high concentrations of HBV particles may be present in the blood (up to 10^{10} particles/mL) HBV DNA polymerase activity, HBV DNA, and HBeAg are characteristic of the viremic stage of hepatitis B, occur concomitantly or shortly after the first appearance of HBsAg during which communicability is highest at this time.

Anti-HBc antibody is against the 27-nm internal core component of HBV High levels of IgM-specific anti-HBc are often detected at the onset of clinical illness. Its appearance indicates the viral replication. Antibody to HBsAg is first detected at a variable period after the disappearance of HBsAg. It is present in low concentrations. Before HBsAg disappears, HBeAg is replaced by anti-HBe, indicating the start of resolution of the disease. Anti-HBe levels often are no longer detectable after 6 months.

HBV chronic carriers

By definition, HBV chronic carriers are those in whom HBsAg persists for more than 6 months in the presence of HBeAg or anti-HBe. HBsAg may continue for years after loss of HBeAg. In contrast to the high titers of IgM-specific anti-HBc detected in acute disease, low titers of IgM anti-HBc are found in the sera of most chronic HBsAg carriers. Small amounts of HBV DNA are usually detectable in the serum as long as HBsAg is present.

The most useful detection methods are ELISA for HBV antigens and antibodies and PCR for viral DNA.

PREVENTION & CONTROL

Vaccine

Since 1982 vaccine for hepatitis B is available. The early vaccine was prepared by purifying and formalin/heat treated HBsAg associated with the 22-nm particles from healthy HBsAg-positive carriers. Recent plasma-derived vaccines have been substituted by recombinant DNA-derived vaccines consisting of HBsAg produced by a recombinant DNA in yeast cells or in continuous mammalian cell lines. The HBsAg expressed in yeast forms particles 15–30 nm in diameter, with the morphologic characteristics of free surface antigen in plasma though the polypeptide antigen produced by recombinant yeast is not glycosylated. The vaccine formulated using this

purified material has a potency similar to that of vaccine made from plasma-derived antigen.

Vaccination

Hepatitis B vaccination is the most effective method in the prevention of HBV and its consequences. Hepatitis B vaccine currently is recommended by the WHO, CDC (Centre's for Disease Control and Prevention), and the Advisory Committee on Immunization Practices for all susceptible, at-risk groups. It involves universal vaccination of infants, routine screening of all pregnant women for HBsAg, post exposure immune prophylaxis of infants born to HBsAg-positive mothers, vaccination of children and adolescents not previously vaccinated, and vaccination of unvaccinated adults at increased risk of infection like commercial sex workers and trans genders.

Response to vaccination in Immunosuppressed groups, like HIV infected, patients on chemotherapy and haemodialysis patients less compared to healthy individuals.

Passive immunization with hepatitis B immune globulin (HBIG) is effective when given soon after exposure. Persons exposed to HBV percutaneous route or by contamination of mucosal surfaces should immediately receive both HBIG and HBsAg vaccine administered simultaneously at different sites to provide immediate protection with passively acquired antibody followed by active immunity generated by the vaccine.

Pregnant women with type B hepatitis can transmit the disease to their infants. The efficiency of hepatitis vaccine and HBIG in averting hepatitis B infection in infants born to HBV-positive mothers is well documented.

The spouses and intimate contacts of persons with acute type B hepatitis are at increased risk of obtaining clinical type B hepatitis so it is essential to inform that these practices might increase the risk of infection or transmission which makes councilling for these patients and vaccination for the high risk groups is important in curtailing the disease.

HEPATITIS C VIRUS

Worldwide there are more than 170 million chronic carriers who are at risk of developing liver cirrhosis and liver cancer. In 1997 the WHO has estimated that about 3% of the world population has been infected with Hepatitis C virus infection, with high prevalence rates of 10% in Africa followed by South America and Asia.

STRUCTURE:

Hepatitis C virus belonging to family Flaviviridae and genus*Hepacivirus* is a positive-stranded RNA virus. Various viruses can be differentiated by RNA sequence analysis into at least six major genotypes (clades) and more than 100 subtypes. The genome is 9.4 kb in size and encodes a core protein, two envelope glycoproteins, and several non-structural proteins.

HCV exhibits genomic diversity, with different genotypes (clades) predominating in different parts of the world. During chronic infections the virus undergoes sequence variation. This complex viral population in a host is referred to as "quasi-species." This genetic diversity is not correlated with differences in clinical disease, although differences do exist in response to antiviral therapy according to viral genotype.

Routes of transmission

HCV is transmitted mainly by direct percutaneous exposures to blood, though in 10–50% of cases the cause of HCV cannot be identified. In approximately decreasing order of prevalence of infection are IV drug users (about 80%), haemophiliacs treated with clotting factor products, recipients of transfusions from HCV-positive donors, chronic haemodialysis patients (10%), persons who engage in high-risk sexual practices, and health care workers (1%). Estimates of vertical transmission of mother-to-child vary from 3% to 10%. HCV infection has been associated with tattooing. Mothers with higher HCV viral loads or coinfected with HIV more frequently transmit HCV. No risk of transmission has been associated with breast feeding.

INCUBATION PERIOD

The average incubation period for HCV is 6–7 weeks. The average time from exposure to seroconversion is 8–9 weeks, and about 90% of patients are anti-HCV-positive within 5 months.

CLINICAL FINDINGS

Most primary infections are clinically mild in which 20–30% have jaundice, 10–20% have only nonspecific symptoms such as anorexia, malaise, and abdominal pain. Most of the times the new infections with HCV are subclinical. The majority (70–90%) of HCV patients develop chronic hepatitis, and many are at risk of progressing to chronic active hepatitis and cirrhosis (10–20%). Diseases related with chronic HCV infections include mixed cryoglobulinemia and glomerulonephritis.

Laboratory diagnosis

Serologic assays are available for diagnosis of HCV infection. Enzyme immunoassays (EIA) detect antibodies to HCV but do not distinguish between acute, chronic, or resolved infection. Anti-HCV antibodies can be detected in 50–70% of patients at onset of symptoms, whereas in others antibody appearance is delayed 3–6 weeks. Antibodies are directed against core, envelope, and NS3 and NS4 proteins and tend to be relatively low in titer. Nucleic acid-based assays (PCR, RT-PCR) can genotype the HCV isolates and detect the presence of circulating HCV RNA and are beneficial for monitoring patients on antiviral therapy.

Prevention and control

Even though several candidate vaccines are under research currently there is no vaccine for hepatitis C .Control measures emphasis on prevention activities that reduce risks for contracting HCV which are screening and testing blood, plasma, organ, tissue, and semen donors; virus inactivation of plasma-derived products; counselling of persons with high-risk drug or sexual practices; implementation of infection control practices in health care and other settings; and professional and public education.

BACTERIAL CAUSES OF STI

NEISSERIA GONORRHOEAE

Neisseria gonorrhoeae infection one of the commonest STIs worldwide in distribution which is estimated to be about 106.1 million new cases worldwide. Gonorrhoea is exclusively transmitted by sexual contact, often by women and men with asymptomatic infections. The infectivity of the organism is such that the chance of acquiring infection from a single exposure to an infected sexual partner is 20–30% for men and even greater for women. The infection rate can be reduced by avoiding multiple sexual partners, rapidly eradicating gonococci from infected individuals by means of early diagnosis and treatment, and finding cases and contacts through education and screening of populations at high risk. Mechanical prophylaxis (condoms) provides partial protection.

Chemoprophylaxis is of limited value because of the rise in antibiotic resistance of the gonococcus.

Neisseria gonorrhoeae

Gonococci is a gram negative intracellular diplococci which ferments only glucose and differ antigenically from the other neisseriae.

Antigenic Structure

N gonorrhoeae is antigenically heterogeneous and capable of changing its surface structures in vitro—and presumably in vivo—to avoid host defenses. Surface structures include the following.

Pili (Fimbriae)

Pili are the hair-like appendages that extends from the surface of the organisms which improves the attachment to host cells and resistance to phagocytosis. Mostly all the strains of *N gonorrhoeae* will be having antigenically different pillins.

Por

Por protein extends through the gonococcal cell membrane.Por proteins are having important impact intracellular killing of gonococci within neutrophils by preventing phagosome-lysosome fusion.

Opa Proteins

Opa proteins plays important role in attachment of gonococci to host cells and attachment of gonococci within colonies

Lipooligosaccharide (LOS)

Gonococci can express more than one antigenically different LOS chain simultaneously. Toxic nature of the gonococcal infections is mainly due to the endotoxic effects of LOS.

The sialyation of terminal galactose of human glycosphingolipids with sialic acid makes the organism resistant to killing by the human antibody-complement system and interferes phagocytic cells binding to the organism.

Antibiotic resistance

Gonococci has many plasmids of which 95% of strains have a small, "cryptic" plasmid of unknown function. Two other plasmids (MW 3.4 x 10^6 and 4.7 x 10^6) comprises genes coding for β-lactamase production making them resistant to penicillin which could be acquired from *Haemophilus* or other gram-negative organisms. They are transmissible by conjugation among gonococci. Insertion of a streptococcal gene tetM coding for tetracycline resistance into the conjugative plasmid has brought the high-level tetracycline resistance in gonococci.

PATHOGENESIS

Mucous membranes of the genitourinary tract, eye, rectum, and throat are the targets of gonococci causing acute suppuration leading to tissue invasion followed by chronic inflammation and fibrosis.

In males, mostly it causes urethritis and sometimes it can be asymptomatic. Urethritis causes yellow, creamy pus and painful urination. If untreated suppuration diminishes and fibrosis occurs, occasionally leading to urethral strictures.

In females, endocervix is site to get affected initially which then spreads to the urethra and vagina, giving rise to mucopurulent discharge. Infection in the fallopian tubes, causes salpingitis, fibrosis, and tubal obstruction causing infertility.

Gonococcal bacteremia leads to skin lesions of the hands, forearms, feet, and legs. Gonococcal tenosynovitis and suppurative arthritis, generally affects knees, ankles, and wrists. Gonococci can be cultured from blood or joint fluid of only 30% of patients with gonococcal arthritis.

Gonococcal endocarditis is a rare yet severe infection. Gonococcal bacteremia is seen frequently in complement deficiency. Gonococcal ophthalmia neonatorum is acquired during passage through an infected birth canal. If untreated the conjunctivitis rapidly progresses to blindness. To prevent

gonococcal ophthalmia neonatorum can be prevented by instillation of tetracycline, erythromycin, or silver nitrate.

Diagnostic Laboratory Tests

Specimens

Pus and secretions are taken from the urethra, cervix, rectum, conjunctiva, throat, or synovial fluid for culture and smear.

Smears

Gram-stained smears of endocervical or urethral exudate shows many intracellular gram negative diplococci within pus cells. These give a presumptive diagnosis. Stained smears of the urethral exudate from men have a sensitivity of about 90% and a specificity of 99%.

Stained smears of endocervical exudates have a sensitivity of about 50% and a specificity of about 95%. Cultures of urethral exudate from men are not necessary when the stain is positive, but cultures should be done for women. Stained smears of conjunctival exudates can also be diagnostic, but those of specimens from the throat or rectum are generally not helpful.

Culture

Immediately after collection, pus or mucus is streaked on modified Thayer-Martin medium (has antibiotics like Vancomycin, 3µg/mL; Colistin, 7.5 µg/mL; Amphotericin B, 1µg/mL; and Trimethoprim, 3µg/mL) and

incubated in 5% CO_2(candle jar) at 37 °C. If immediate incubation is not possible, the specimen should be placed in a CO_2-containing transport-culture system. Forty-eight hours after culture, the organisms can be quickly identified by their appearance on a Gram-stained smear, by oxidase positivity, and by coagglutination, immunofluorescence staining, or other laboratory tests. The species of sub cultured bacteria may be determined by fermentation reactions RCUT (rapid carbohydrate utilisation test).

Gonococci generally produce smaller colonies than those of the other neisseriae. Gonococci that require arginine, hypoxanthine, and uracil tend to grow most slowly on primary culture. Gonococci isolated from clinical specimens or maintained by selective subculture like modified Thayer Mayer medium will have typical small colonies containing piliated bacteria. On nonselective subculture as in chocolate culture larger colonies containing nonpiliated gonococci are formed. Opaque and transparent variants of both the small and large colony types also occur.

Nucleic Acid Amplification Tests

Several FDA-cleared nucleic acid amplification assays are available for direct detection of *N gonorrhoeae* in genitourinary specimens. Nucleic Acid Amplification Tests have excellent sensitivity and specificity in symptomatic, high-prevalence populations. Advantages of these Nucleic Acid Amplification Tests is better detection, more rapid results, and the ability to use urine as a

specimen source. Disadvantages include poor specificity of some assays due to cross reactivity with nongonococcal*Neisseria* species. These tests are not suggested for use for the diagnosis of extragenitalgonococcal infections.

Serology

Serum and genital fluid contain IgG and IgA antibodies against gonococcal pili, outer membrane proteins, and LPS. Some IgM of human sera is bactericidal for gonococci in vitro.These antibodies can be detected by immunoblotting, radioimmunoassay, and ELISA tests. But theseassays are not useful as diagnostic aids for many reasons like gonococcal antigenic heterogeneity, the delayed antibody development in acute infection; and a high background level of antibodies in the sexually active population.

Immunity

Repeated gonococcal infections are common. Protective immunity to reinfection does not appear because ofgonococcal antigenic heterogeneity. Antibodies like IgA and IgG can be demonstratedon the mucosal surfaces, they either are highly strain-specific or have little protective ability.

Treatment

Development and the widespread use of penicillinhas increased the gonococcal resistance to penicillin so that many strains now require high concentrations of penicillin G for inhibition. Penicillinase-producing *N*

gonorrhoeae (PPNG) also have increased in prevalence. High-level resistance to tetracycline also occurring due to chromosomally mediated resistance to tetracycline. Spectinomycin resistance and fluoroquinolones resistance has been noted.

Gonococcalophthalmianeonatorum is prevented by local application of 0.5% erythromycin ophthalmic ointment or 1% tetracycline ointment to the conjunctiva of newborns. As silver nitrate solution is difficult to store and can causes conjunctival irritationit has mostly been substituted by use of erythromycin or tetracycline ointment.

As the problems with antimicrobial resistance in *N gonorrhoeae,* the US Public Health Service recommends that uncomplicated genital or rectal infections be treated with ceftriaxone given intramuscularly as a single dose.

TREPONEMAPALLIDUM

STRUCTURE

Treponemapallidum is an actively motile slender spirals rotating steadily around their endoflagella measuring about 0.2 μm in width and 5–15 μm in length. The spiral coils are regularly spaced at a distance of 1 μm from one another. The long axis of the spiral is usually straight but may occasionally bend, so that the organism forms a complete circle sometimes and returningback to its normal straight position.

The spirals are so thin that they are not easily seen unless immunofluorescent stain or dark field illumination is used.it can be visualised by staining with silver impregnation method.

Genome

The *T pallidum* genome is a circular chromosome of about 1138kbp which is small when compared with other bacteria and also they don't have transposable elements, which makes their genome highly conserved and that explains its constant susceptibility to penicillin. There are only few genes involved in energy production and synthesis of nutrients, which makes *T pallidum* to obtain these from the host.

Antigenic Structure

Since T *pallidum* cannot be cultured in vitro the characterization of its antigens is limited. The outer membrane surrounds the periplasmic space which has the endoflagella and the peptidoglycan-cytoplasmic membrane complex. The lipids seems to bind the proteins to the cytoplasmic or outer membranes and keep the proteins unreachable to antibodies.

The endoflagella are composed of three core proteins similar to other bacterial flagellin proteins, plus an unrelated sheath protein. *T pallidum* subspecies *pallidum*has an enzyme hyaluronidase whichdisruptions the hyaluronic acid in the ground substance of tissue responsible for the

invasiveness of the organism. Approximately more than 100 protein antigens have been recognised. The protein profiles of *T pallidum* (all the subspecies) are indistinguishable; Cardiolipin is an important component of the treponemal antigens.

Culture

Pathogenic *T pallidum* has never been cultured continuously on any artificial media. Only the non-pathogenic treponemes Reiter strain, can be cultured anaerobically in vitro.

Growth Characteristics

T pallidum is a microaerophilic organism which survives best in the environment containing 1–4% oxygen. The non-pathogenic treponemes Reiter strain grows on a well-defined medium of 11 amino acids, vitamins, minerals,albumin, salts, and serum.

When kept in proper suspending fluids and reducing substances, *T pallidum* may remain motile for 3–6 days at 25 °C. In whole blood or plasma stored the organisms remains viable for at least 24 hours when stored at 4 °C, which is of potential importance in blood transfusions.

Pathogenesis, Pathology, & Clinical Findings

Acquired Syphilis

Natural infection with *T pallidum* is restricted to the human host and transmitted by sexual contact. The infectious lesion is on the skin or genital mucous membranes. However in 10–20% of cases, the primary lesion is intrarectal, perianal, or oral. It can penetrate intact mucous membranes and even it can enter through a break in the epidermis.

T pallidum multiply locally at the entry site, and spread to nearby lymph nodes and then reach the bloodstream. In 2–10 weeks after infection, a papule develops at the site of infection and breaks down to form an ulcer with a clean, hard base which is called the "hard chancre". The inflammation is characterized by abundant of lymphocytes and plasma cells. This "primary lesion" always heals spontaneously, but 2–10 weeks later the "secondary" lesions appear. Secondary lesions consist of a red maculopapular rash anywhere on the body, including the hands and feet, and moist,condylomas in the anogenital region, mouth and axilla. Complications like syphilitic meningitis, chorioretinitis, hepatitis, nephritis, or periostitis also occurs. The secondary lesions also recede spontaneously. Both primary and secondary lesions are highly infectious and rich in spirochetes. Contagious lesions may reappear within 3–5 years after infection, but after that the individual is not infectious. *T pallidum* infection may remain subclinical, and the patient may go through the primary or secondary

stage or even both without any symptoms or signs and may develop to tertiary lesions.

Early syphilitic infection progresses spontaneously to complete cure without treatment in about 30% of cases. In another 30%, the infection remains latent (known only by positive serologic tests). In the remaining, the disease progresses to the "tertiary stage," characterized by the development of gummas in skin, bones, and liver and degenerative changes involving the central nervous system (meningovascular syphilis, paresis, tabes) and cardiovascular system (aortitis, aortic aneurysm, aortic valve insufficiency). In all tertiary lesions, finding treponemes are very rare, and the exaggerated tissue response must be attributed to hypersensitivity to the organisms. Though, the treponemes can rarely be seen in the eye or central nervous system in late syphilis.

Congenital Syphilis

Vertical transmission from the pregnant syphilitic mother to the foetus through the placenta happens in the 10th to 15th weeks of gestation. Some of the infected foetuses die resulting in miscarriages; others are stillborn at term. The infected foetuses born lives but develop the signs of congenital syphilis in childhood like interstitial keratitis, Hutchinson's teeth, saddle nose, periostitis, and a range of central nervous system anomalies. Proper treatment of the mother during pregnancy prevents congenital syphilis.

Diagnostic Laboratory Tests

Demonstration of antibodies against *T pallidum* by indirect immunofluorescence, immobilizing and killing live motile *T pallidum* and fixing complement in the presence of a suspension of *T pallidum* or related spirochetes is the diagnostic tests available. Reagin is a mixture of IgM and IgG antibodies directed against the cardiolipin-cholesterol-lecithin complex andgives positive complement fixation and flocculation tests with aqueous suspensions of cardiolipin extracted from normal mammalian tissues. Both reagin and antitreponemal antibody are used for the serologic tests of syphilis.

Specimens

Specimens include tissue fluid expressed from early skin/mucosal surface lesions for demonstration of spirochetes; blood serum for serologic tests.

Darkfield Examination

A drop of tissue fluid or exudate is placed on a slide and a cover slip pressed over it to make a thin layer and examined under oil immersion with dark field illumination for motile spirochetes.

Immunofluorescence

The tissue fluid or exudate is spread on a glass slide, air dried, fixed, stained with a fluorescein-labelled antitreponeme serum, and examined with immunofluorescence microscopy for typical fluorescent spirochetes.

Serologic Tests for Syphilis (STS)

These tests use either nontreponemal or treponemal antigens.

Nontreponemal Antigen Tests

The antigens used in these tests are lipids extracted from normal mammalian tissue. The purified cardiolipin from beef heart is a diphosphatidylglycerol. Lecithin and cholesterol are added to increase reaction with syphilitic "reagin" antibodies. The VDRL (Venereal Disease Research Laboratory) and RPR (rapid plasma reagin) tests are nontreponemal antigen tests used most generally. The toluidine red unheated serum test (TRUST) also is available. All of the tests are based on the fact that the particles of the lipid antigen remain spread with normal serum but flocculate when combining with reagin.

The VDRL flocculation testrequires microscope for detection, whereas the RPR and TRUST have the colored particles like carbon and can be read with naked eye. Results develop within a few minutes, particularly if the suspension is agitated.

Positive VDRL or RPR tests becomes positive after 2–3 weeks of infection and are positive in high titer in secondary syphilis. Positive VDRL or RPR tests return to negative in 6–18 months after effective treatment of syphilis. The VDRL test can done in cerebrospinal fluid and becomes positive after 4–8

weeks of infection. Even though the reagin antibodies do not cross blood brain barrier and reach the cerebrospinal fluid from the bloodstream but are perhaps formed in the central nervous system in response to syphilitic infection.

The flocculation tests can give quantitative results by estimating of the amount of reagin present in serum with twofold dilutions of serum and expressing the titer as the highest dilution that gives a positive result. Quantitative results are valuable in establishing a diagnosis especially in neonates and in monitoring the effect of treatment.

Nontreponemal tests can give biological false-positive results due to the occurrence of reagin antibody in many human disorders. Important among them are malaria, leprosy, measles, infectious mononucleosis, vaccinations,systemic lupus erythematosus, polyarteritis nodosa, rheumatic disorders and other conditions.

Treponemal Antibody Tests

Fluorescent Treponemal Antibody (FTA-ABS) Test

It is an indirect immunofluorescence in which the killed *T pallidum*, patient's serum and labelled antihuman gamma globulin are used. FTA-ABS has good specificity and sensitivity for syphilis antibodies if the patient's serum has been absorbed with sonicated Reiter spirochetes prior to the FTA test. The FTA-ABS test is the first to become positive in early syphilis, is usually positive

in secondary syphilis. This test usually remains positive many years after effective treatment making it unable to judge the efficacy of treatment. The presence of IgM FTA in the blood of new-borns is good evidence of in congenital syphilis.

Treponemapallidum-Particle Agglutination (TP-PA) Test

These are the *T pallidum*hemagglutination **(TPHA)** and microhemagglutination for *T pallidum* **(MHA-TP)** tests similar to the FTA-ABS test in specificity and sensitivity. Particles are sensitized with *T pallidum* subspecies *pallidum* antigens. The test is performed with diluted serum. Antibodies against *T pallidum* react with the sensitized particles. A mat of agglutinated particles indicates a positive result.

Immunity

A person with active or latent syphilis or yaws seems to be resistant to superinfection with *T pallidum.*Conversely, if early syphilis or yaws is treated effectively and the infection is eradicated, the patient again becomes completely susceptible.

Treatment

Penicillin is the treatment of choice. Penicillin in concentrations of 0.003 unit/mL has definite treponemicidal activity. Single injection of benzathine penicillin G intramuscularly can treat Syphilis of less than 1 year

duration. If a person is allergic to penicillin then other antibiotics like tetracyclines and erythromycin can be substituted. A typical Jarisch-Herxheimer reaction may occur within hours after treatment is begun which is due to the release of toxic products from dying or killed spirochetes.

BACTERIAL VAGINOSIS

Previously known as non-specific vaginitis, anaerobicvaginosis, Gardernerella vaginosis or haemophilus vaginalis vaginitis but later after 1983 the term bacterial vaginosis become popular as this condition is a combination of overgrowth of certain bacteria and the absence of inflammatory response. It is the commonest cause of vaginal discharge of women of child bearing age group.it is a clinical syndrome with the symptoms of increased vaginal discharge with foul smelling fishy odour which is more offensive after sexual intercourse

ETIOLOGICAL AGENTS

Gardernerella vaginalis, Prevotella spp.,Peptostreptococci,Mobiluncus spp., like Mobilun cuscurtisii which are small curved gram variable organisms and Mobiluncus mulieris which is long curved ,gram negative organisms and Mycoplasma hominis are closely associated with bacterial vaginosis.Eventhough these organisms are found in normal vagina but in BV these organisms will increase 100-1000 fold[21].

Pathology

The organisms will adhere to the squamous epithelial cells and will give them studded appearance .this type of studded epithelial cell is called as 'clue cells. These organisms produce amines which increases the vaginal pH from from 4.5 to 5.0 or greater.

Predisposing factors

Its prevalence is more among the sexually active women attending the STD clinics and women using intrauterine devices[21].

Diagnosis:

The accepted standard for the diagnosis of BV has been the Amsels criteria which include thin homogenous vaginal discharge, elevated vaginal pH, presence of amines detected by fishy odour after the addition of KOH to the discharge and the presence of clue cells. Since in this method vaginal examination is needed and fishy odour is a subjective feature.

Alternative to this method is the Nugent's scoring in which the grading or scoring of the gram stained vaginal smear to detect the changes in vaginal ecology .Quantification of the number of different type bacteria like lactobacilli, Gardernerella ,Bacteroides and mobiluncus is done and the score for each of these is combined and grading is done.

TREATMENT

Metronidazole is the drug of choice is 400mg for 5 days and the partner will have to be treated simultaneously. Topical clindamycin 2% has been also used twice daily for 7 days.

SEQUELAE

Women with BV who are pregnant have increased the risk of late miscarriage and preterm birth.

PARASITIC CAUSES OF STI

TRICHOMONIASIS

T vaginalis is a common parasite of both males and females. Infection rates vary greatly but may be quite high (40% or higher). Transmission is by sexual intercourse, but contaminated towels, examination instruments, and other objects acts as fomites and may be responsible for some new infections. Infants may be infected during birth.

STRUCTURE

T vaginalis is pear-shaped, with a short undulating membrane lined with a flagellum and four anterior flagella. It measures about 10 x 7 μm, though its length may vary from 5 to 30 μm and its width from 2 to 14 μm. The organism moves with a characteristic wobbling and rotating motion. The non-pathogenic trichomonads like Trichomonas*hominis* and *Trichomonastenax,* cannot readily

be distinguished from *T vaginalis* when alive. For all practical purposes, trichomonads found in the mouth are *T tenax;* in the intestine, *T hominis;* and in the genitourinary tract (both sexes)*T vaginalis.*

Pathogenesis, Pathology, & Clinical Findings

In females, the infection is normally limited to vulva, vagina, and cervix; it does not usually extend to the uterus. The mucosal surfaces may be tender, inflamed, eroded, and covered with a frothy yellow or cream-colored discharge.

In males, the prostate, seminal vesicles, and urethra may be infected. Signs and symptoms in females, in addition to profuse vaginal discharge, include local tenderness, vulval pruritus, and burning. About 10% of infected males have a thin, white urethral discharge.

Diagnostic Laboratory Tests

Specimens and Microscopic Examination

Wet mount of vaginal or urethral secretions or discharge shows characteristic motile trichomonads. Dried smears may be stained with Giemsa or other stains.

Culture

Trichomonas Modified CPLM Medium Base with addition of horse serum and antibiotics is recommended for cultivation of Trichomonas species. It contains L-Cysteine, Pepticdigest, liver digest and maltose with antibiotics like penicillin, streptomycin and antifungals Nystatin.Culture of vaginal or urethral

discharge, of prostatic secretion, or of a semen specimen may reveal organisms when direct examination is negative. Simplified trypticase serum is usually used for semen cultures. *T vaginalis* grows best at 35–37 °C under anaerobic conditions, less well aerobically. The optimal pH for growth in vitro (5.5–6.0) suggests why vaginal Trichomoniasis is more severe in women with higher than normal vaginal pH

Treatment

Successful treatment of vaginal infection requires destruction of the trichomonads, for which topical and systemic metronidazole is best. Partner treatment is also important in complete treatment regimen. Condoms should be used during intercourse until the infection is eradicated in both partners.

FUNGAL CAUSES OF SEXUALLY TRANSMITTED INFECTIONS.

CANDIDIASIS

Candidal vulvovaginitis is believed to be the most frequent or second most frequent cause of vaginal infection, depending upon the geographical area and its incidence is estimated in the range of 5 to 20 percent[21].

The first known description of candida infection, oral candidiasis (Thrush) in two patients with other underlying disease, may be found in Hippocrates 'Epidemics' from the fourth century BC.Even though the existence of candidiasis from very beginning two major medical events had increased the

interest in the fungal diseases particularly candida infections. The first was the introduction of broad spectrum antibiotics which caused the imbalance of hosts normal flora in favour of fungi upon which these antibiotics have no action and the another one is the increased prevalence of immunosuppressed patients due to chemotherapy and diseases like AIDS in the recent few decades both of these events had increased the incidence of candida infection in general and also the non C.albicans[21] .Along with these the increased use of broad spectrum antifungals like azoles had changed the decreased prevalence of C.albicans as the etiological agent of candidiasis and increased the proportion of non-albicans species such as C.glabrata or C.krusei

ETIOLOGICAL AGENT

The genus candida includes about 200 species out of which about 20 have been associated with infections in humans and animals. The main pathogenic organism is the candida albicans and the candida non albicans like candida glabrata, candida tropicalis, candida krusei, candida parapsilosis and candida dubliniensis.

CLINICAL FEATURES

Vulvovaginal candidiasis includes the symptoms of Vulvovaginal pruritis and vaginal discharge which is thick and curd like. Erythema of the Vulvovaginal mucosa is also seen.in males candidiasis manifests as balanitis and erythema on the penis.

LABORATORY DIAGNOSIS

DIRECT EXAMINATION

Specimen from oral and fungal candidiasis is taken by swab and the specimen is need not to be treated with keratinolytic agents. Both the wet mount and gram, methylene blue or Giemsa stain can be done.

Microscopic examination of specimens reveals presence of budding yeast cells, pseudohyphae and /or hyphae. The demonstration of hyphal elements in clinical specimens is important as it is a indicator of infection since the mouth and vagina can be normally colonised by candida spp., however another fact also to be taken in consideration is that candida glabrata, a important non albicans species of vaginal candidiasis does not produce any hyphae or pseudohyphae in clinical specimens.

CULTURE

Sabourauds dextrose agar supplemented with antibiotics like chloramphenicol, Gentamicin and /or Tetracycline to prevent the bacterial overgrowth and incubated at28^0C or/and at 37^0C the colonies will be apparent within 24hrs or up to 3 days maximum.

Germ tube test

The ability of C.albicans or C.dubliniensisblastospores to produce germ tubes in serum incubated at 37^0C for 2 hours offers a reliable and easy method of identification.

Speciation of Candida

The macroscopic appearances of the colonies of candida and non albicans are almost similar with only few differences like in candida krusei the colonies will be more dry creamy colonies compared to candida albicans which have creamy pasty colonies but these are difficult to interpret and have subjective variation.

CHROMagar system

This method uses principle that reaction between the specific enzymes of the different species and chromogenic substances present in the culture medium will produce the differently coloured colonies.

PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISATION

Tests based on the pattern of utilisation of specific carbohydrate and nitrogen substances are used for speciation which includes Wicerham and Burton method and auxanographic technique and also by the commercial kits.

MOLECULAR METHODS

The common molecular probes used for the molecular studies like PCR, restriction enzyme analysis and RNA profiling are ribosomal DNA primers including large and small rRNA subunits and ERG11 gene which is involved in ergosterol biosynthesis

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY DESIGN

Prospective Study

PLACE OF STUDY

1. Department of Microbiology, Stanley medical College, Chennai.
2. Department of Sexually Transmitted Diseases, Stanley medical College and Hospital, Chennai

Study Period:

September 2013 to September 2014.

SAMPLE SIZE

The sample size was determined using Open Epi Version 2.3.1 with a power of 80% and confidence level of 0.05. The estimated sample size was 196 considering the attrition rate 2 % a total sample of 200 was used for study enrollment**.**

INCLUSION CRITERIA

1. Trans genders involved in the commercial sex works

2. Male Homosexuals

3. Commercial Sex workers

EXCLUSION CRITERIA

1. Patients who are treated for any of the sexually transmitted infections with antibiotics.

2. Women aged > 18 yrs attending STD clinic and from Referral Centres both symptomatic and asymptomatic

3. Men aged >18 yrs attending STD clinic and from Referral Centres both symptomatic and asymptomatic

4. Unmarried women, pregnant women and Women with vaginal bleeding

Ethical Consideration

Ethical and research clearance was obtained from the Ethical committee Stanley Medical College. Permission to conduct the study was sought from the respective hospital department authorities. Informed consent was obtained from the patients before enrolment in to the study.

Statistical Analysis

The collected data was analyzed with SPSS 16.0 version. To describe about the data descriptive statistics frequency analysis, percentage analysis were used for categorical variables and for continuous variables the mean and S.D were used. Receiver operating characteristic (ROC) curves were drawn to find out area under the curve (AUC) so as to achieve the highest average sensitivity and specificity. To find the significance in categorical data Chi-Square test was

used. In both the above statistical tools the probability value .05 is considered as significant level

SPECIMEN COLLECTION:

Method of collection of specimen:

1) Urethral swab [22, 23]:

The specimen was collected after wearing sterile gloves and 1 hour after the patient has urinated. After retracting the prepuce, tip of the meatus was cleaned with normal saline and if purulent discharge (pus) from urethra was seen, it was collected using a sterile cotton wool swab or loop. If no discharge was seen, milking of the urethra was done towards the orifice to obtain the discharge. Six swabs were taken – one for gram stain, one for direct plating into Modified Thayer Martin medium &chocolate agar for gonococcus culture, one for inoculation into Sabourads dextrose agar for Candida culture, one for wet mount & one for inoculation into Diamonds medium for *Trichomonas vaginalis* culture and one swab placed in 70% ethanol test tube for viral isolation. If discharge was not seen even after milking, a sterile thin cotton wool swab inserted 2-3 cms into the urethra and rotated for 5-10 seconds to gently scrap the mucosa and then inoculated into Chocolate agar & Modified Thayer Martin medium for gonococcus culture.

THROAT SWAB

COLLECTION AND TRANSPORT OF THROAT SWABS[25]

It was made sure that the patient was not treated with antibiotics or antiseptic mouth-washes (gargles) 8 hours before swabbing.

1. In a good light and using the handle of a tongue depressor the tongue was depressed and inside of the mouth was examined. Any inflammation, membrane, exudate, or pus are noted

2. Swabbing done in the tonsils, tonsillar crypts, peritonsillar area and posterior pharyngeal wall using a sterile cotton wool swab. Swab was taken without contaminating the swab with saliva and placed in the sterile test tube.

Four swabs were taken – one for gram stain, one for direct plating into Modified Thayer Martin medium & chocolate agar for gonococcal culture and one swab placed in 70% ethanol test tube for viral isolation.

Collection of cervical specimens from female patients

Sterile vaginal speculum was used to examine the cervix and the specimen was collected.

Speculum was moistened with sterile warm water instead of lubricating gel as it may be bactericidal, and inserted into the vagina. Cervix was cleaned using a swab moistened with sterile physiological saline a sterile Dacron swab is passed 20–30 mm into the endocervical canal and gently rotated against the

endocervical wall to obtain a specimen. Four swabs were taken – one for gram stain, one for direct plating into Modified Thayer Martin medium & chocolate agar for gonococcus culture and one swab placed in 70% ethanol test tube for viral isolation.

Collection of vaginal discharge

Wet mount to detect motile T. vaginalis:

Using a sterile Dacron swab specimen is collected from the vagina. And the sample of the exudate is transferred in to a microscope slide to which a drop of normal saline is added and mixed and then covered with a cover glass.

Direct smear for Gram staining to detect Candida and examine for clue cells

Using a sterile Dacron swab specimen was collected from the vagina. Sample of the exudate is transferred to a microscope slide and then spreaded to make a thinsmear. Smear was air dried and heat fixed for gram staining.

Rectal swabs:

Specimen was collected using a sterile cotton wool swab. After the application of proctoscope moist Swab was inserted in to the rectum for about 2 to 4 cm .swabs taken avoiding unnecessary contamination of the specimen with bacteria from the anal skin. Swabs with heavy faecal contamination is discarded. Four swabs were taken – one for gram stain, one for direct plating

into Modified Thayer Martin medium & chocolate agar for gonococcus culture and one swab placed in 70% ethanol test tube for viral isolation.

3) Blood:

5 ml of blood collected from each patient and serum was separated either by keeping the sample containing test tube in 45° slanting position in refrigerator at 4-8°C for 4-6 hours or by centrifuging at 1500 rpm for 5-10 min. Serum was stored in sterile container at -20°C.Then the serum was tested for HIV as per NACO guidelines[23], RPR for Syphilis, IgM and IgG antibody Elisa for Herpes Simplex virus 2, Rapid card test for Hepatitis B & C.

SPECIMEN PROCESSING: IDENTIFICATION OF ORGANISMS

1) *Neisseria gonorrhoeae-*

a) Microscopy

With one swab, on a clean grease free slide, smear was prepared by rolling the swab in single direction. One directional smearing technique minimizes distortion and breakage of Polymorphonuclear leukocytes (PMNL) and thereby preserves the characteristic intracellular appearance of this microorganism. The smear was air dried and methanol fixed. Gram stain was done using saffranin as counterstain and examined under light microscope after putting a drop of cedar wood oil on the slide.

Reading:

The smear was examined for epithelial cells, Polymorphonuclear leucocytes (pus cells), organisms and their location whether extracellular or intracellular. The gonococci are intracellular; bean shaped and are usually arranged in pairs, 0.8µm x 0.6µm in size. They are Gram negative in reaction and are stained pink along with the nuclei and protoplasm of pus cells. The slide was examined for at least 2 minutes before declaring as negative for gonococci.

b) CULTIVATION:

For inoculation of *Neisseria gonorrhoeae* culture, specimen were usually collected with Dacron or rayon swabs. Since calcium alginate or some cotton swabs may be toxic to Gonococci, they should be used only if the specimens were inoculated immediately into culture media. Media for isolation of *Neisseria gonorrhoeae* – Two types of media were used. One is non selective media like Chocolate agar. Other is selective media like modified Thayer Martin Medium with VCNT (Vancomycin-inhibit gram positive bacteria, Colistin-inhibit gram negative bacteria, Nystatin-inhibit yeasts and moulds, Trimethoprim inhibit swarming of Proteus) or Modified New York City Medium with VCNT . Media for isolation of *Neisseria gonorrhoeae* was kept at room temperature before inoculation and should not be excessively dry or moist. If excessive moisture present, plates are placed upside down in ajar in an incubator at 35°C for 20-30 minutes.

Method for inoculation:

The specimen was inoculated into the culture plate by rolling the swab on the surface of the plate in a "Z" pattern, then cross streaked with a sterile platinum/nichrome loop. The inoculated plates were kept in the upper part of candle jar after keeping the moist cotton ball/ wet paper towels inside the jar to provide >70% humidity and a lighted non colored candle in upright position in the lower part of the candle jar to provide 5% CO2.The lid of the jar was closed and the jar was kept in the incubator at 35 to 37°C after the candle was put off. The plates were examined after 18-24 hours and incubated for another 24 hours if there was no growth. The plates were examined after 48 hours. If no growth, reported as such. Small pin point, 0.5 to 1 mm in diameter, grey to white in color, smooth, translucent, raised convex colonies suggests Neisseria.

***Trichomonas vaginalis*:**

a) MICROSCOPY

Adrop of discharge or urine sediment was put on a clean grease free microscope slide. One drop of normal saline was mixed with sediment and a coverslip was placed over it. The slide was observed first under 10 x magnification. Any field which shows the suspected organism is then seen under 40 x magnification of light microscope.

Reading:

Trichomonads are 15 μm in size, they have a pyriform shape with an anterior tuft of flagella and a lateral undulating membrane, the parasite moves actively, showing jerky motility, Centrifuged samples do not show motility because the flagella are detached during centrifugation.

b) Staining:

On a clean grease free microscope slide smear was prepared from sediment. Then the smear was allowed to dry in air, fixed with methanol and stained with Giemsa stain. Stained smear was observed under oil immersion.

c) **Culture:**

Culture is gold standard for the diagnosis of Trichomoniasis. The specimen was put in Diamond's medium. The inoculum was placed at the bottom of the tube. The tube was incubated at 37º C. The culture was observed from second day onwards. The tubes were incubated for 7 days before declaring it as negative. For culture identification a wet mount was prepared from the culture and observed under microscope for the motile *Trichomonas vaginalis*.

Candida species:

a) **Gram Staining:**

A smear was prepared on a clean microscope slide and heat fixed. Then stained by Gram's Method. Observed under oil immersion.

Reading:

Gram positive budding yeast cells and pseudohyphae**.**

b) Culture:

The sample was inoculated on two Sabouraud's dextrose agar slopes. One slope was incubated at room temperature (25-27ºC). Pasty, opaque, cream colored colonies grow in 24-48 hours. The other slope was incubated at 37ºC gives faster growth. Grams stain showing the presence of budding yeast cells confirmed the diagnosis.

c) Germ tube test:

Few colonies were inoculated into 0.5 ml of pooled human serum. Incubated at 37ºC for 2 hours in incubator. After 2 hours 2 drops of suspension was taken on a clean slide and mount with a coverslip. It was observed under 40 x objective. Observation of germ tube confirms the presence of *Candida albicans*. A germ tube is a short, lateral hyphal extension (filament) of the yeast cell and is not constricted at the base.

d)SPECIATION

Carbohydrate assimilation test:

A sterile Petri dish is taken and divided into four quadrants by marking the back of the plate with a glass marking pencil. About 4ml of Basal medium agar is melted and poured .About 2ml of pure yeast colonies suspension

(approx. 10^6 colonies/ml) is prepared and each quadrant of the plate is labelled with the appropriate initial of the carbohydrate to be used. With the help of a sterile cotton wool swab the agar surface is inoculated with the yeast suspension and the appropriate carbohydrate solution is dropped on each quadrant with a separate dropper. The plate is incubated the plate at 25 – 30^0C for 24 to 72 hours.

Reading:

A growth ring of the yeast and a change in indicator color from purple to yellow – positive test. Carbohydrate is assimilated.

No growth and no change in the indicator color – Negative test. The isolate lacks enzymes to assimilate that particular carbohydrate.

Bacterial vaginosis

The collection of material for diagnosis is ideally performed during a comprehensive pelvic examination using a speculum. At the time of speculum examination, an evaluation of the nature of the discharge is made, specimen from the lateral vaginal wall and posterior fornix was taken with a sterile swab.

The classical BV discharge is thin, homogeneous and grey/yellow in color. However, absence of the classic discharge does not rule out disturbed vaginal flora. Two basic methods of diagnostic testing can be used: laboratory based and clinical 'bedside' testing. For the purposes of laboratory based testing,

the swab was rolled across a slide and the material allowed to air-dry. In the laboratory, the smear should be heat-fixed and Gram-stained.

In the methodology by Nugent et al[26], the swab was obtained from the lateral vaginal wall and rolled on a glass slide. The smears were then heat fixed and Gram stained using safranin as the counterstain. The smear was then evaluated for the following morphotypes under oil immersion (1000x magnification): large Gram-positive rods (lactobacillus morphotypes), small Gram-variable rods (*G vaginalis*morphotypes), small Gram-negative rods (*Bacteroides* species morphotypes), curved Gram-variable rods (*Mobiluncus* species morphotypes) and Gram-positive cocci. Although Gram-positive cocci are not part of the scoring system, some laboratories will report them if they are present in significant numbers. Increased numbers of Gram-positive cocci are not part of the pattern of the normal vaginal flora. A score of zero to three is considered to be normal, four to six is considered intermediate and seven to ten is defined as Bacterial Vaginosis.

Intermediate vaginal flora is reported to the clinician for management based on the clinical context. Thirty two per cent of patients with an intermediate score will proceed to BV and 30% to normal flora. Even though clinical methodology is useful because it allows for an immediate answer in certain urgent clinical situations, but the Gram stain method appears to be more accurate.

NUGENT'S SCORING

S.No	Organism morphotypes	Number/oil immersion field	score
1	Lactobacillis-like(parallel-sided, gram positive rods)	>30 5-30 1-4 <1 0	0 1 2 3 4
2	Mobiluncus–like(curved, gram negative rods)	>5 1-4 0	2 1 0
3	Gardernerella/bacteriods like(tiny,gramvariable coccobacilliandrounded ,pleomorphic,gramnegative rods with vacuole)	>30 5-30 1-4 <1 0	4 3 2 1 0

0-3 normal

4-6 intermediate, test to be repeated later

7-10 bacterial vaginosis

CULTIVATION

Cultures for *G. vaginalis* should not be used to diagnose bacterial vaginosis because 36–55% of women without bacterial vaginosis will harbor this organism as part of normal flora. Although culture is not recommended for diagnosis of BV, the primary isolation of *G. vaginalis* from specimens containing mixed flora can be performed by using a selective, enriched human blood agar medium containing the antibiotics, colistin, and nalidixic acid.

HBT (Human Blood Tween 80) Bilayer Medium was composed of a basal layer of 7 ml of CNA agar base, with amphotericinB (2pg/ml) added after autoclaving was performed, and a 14ml over layer of the same composition plus 5% human blood and 0.0075% Tween 80 (BBL) was added to both layers after autoclaving was performed. It is for the selective isolation and differentiation of Gardnerella vaginalis. Discharge taken from the vaginal discharge is swabbed in the plate and incubated anaerobically at 37^0C in 5 to 10% Co2 for 72 hours as the 72 hours incubation increases the isolation rate of the organism[27]. The anaerobic environment will enhance the hemolytic property of the organism. The greyish white colonies surrounded by zones of diffuse hemolysis indicates growth.

Serological tests

Immunochromatography Rapid test for HBsAg

The HEPA™ Strip HBsAg test is a rapid, qualitative, one-step two-side sandwich immunoassay based on the immunochromatographic sandwich principle. The method employs a unique combination of monoclonal-dye conjugate (colloidal gold) and polyclonal solid phase antibodies to selectively identify Hepatitis B Surface antigen of Hepatitis B viral infection with a high degree of sensitivity.

PROCEDURE:

The specimen and Hepa™ Strip was brought to room temperature prior to testing. 4-6 drops of serum sample is added into the sample well avoiding overflowing and the reaction was proceeded until the appearance of positive line and control line.

The result was red after 20 minutes.

INTERPRETATION OF RESULTS:

POSITIVE: Distinct purple line formed at the test zone marked 'T' (test line) and the control Zone marked 'C' (control line) the test result was positive, indicating that the sample contains Hepatitis B Antigen.

NEGATIVE: Distinct purple line formed only at the control zone marked 'C'

(Control line) the test result was negative.

ONE STEP ANTI HCV TEST

SD BIO LINE HCV test is a immunochromatographic rapid test for the qualitative detection of antibodies specific to HCV in human serum, plasma or whole blood. It is a One-step qualitative immunochromatographic assay which uses the recombinant HCV Core, NS3, NS4, NS5 Ag used as capture materials

Procedure

10µl of serum sample is taken with a pipette and added to the well and then 4 drops of assay diluent provided with the kit is added to the well. The card was red after 20 minutes.

INTERPRETATION OF RESULTS:

POSITIVE: Distinct purple line formed at the test zone marked 'T' (test line) and the control Zone marked 'C' (control line) the test result was positive, indicating that the sample contains Hepatitis C Antigen.

NEGATIVE: Distinct purple line formed only at the control zone marked 'C' (Control line) the test result was negative.

Herpes Simplex Virus 2 –IgM ELISA from serum samples:

IgM Capture ELISA kit from Diagnostic Bioprobes Italy

Principle:

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody.

Procedure:

10 µl of sample was diluted by adding 1mL sample diluent in a dilution tube and then mixed with vortex to diluted sample. The required number of Microwells on holder were placed. A1 is left empty for Blank sample. 100 µl of NC, 100 µl of Calibrator in duplicate were dispensed into respective wells. 100 µl of PC was dispensed in single. Then 100 µl of samples were dispensed in respective wells. Wells are checked for blue color except A1. The strips were covered and incubated for 60 min at 37°C. Wells were washed for 5 cycles. 100 µl of Ag/Ab Immunocomplex dispensed into each well except blank well. The strips were covered and Incubated for 60 min at 37°C.Washed for 5 cycles. 100 µl of Chromogen/substrate mixture dispensed into each well including A1. Incubated for 20 min at 25°C. 100 µl of Sulphuric acid as stopping solution was dispensed into all the wells. PC & Positive samples turned from blue to yellow. OD value measured at 450nm filter, 620-630 nm (background substraction).

Herpes Simplex Virus 2 –IgG ELISA from serum samples:

IgM Capture ELISA kit from Diagnostic Bioprobes Italy

Principle:

The assay is based on the principle of "IgG capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgG antibody.

Procedure:

Microplates in the kit are coated with synthetic HSV2 specific glycoprotein G or gG.To the 10 µl of sample 1mL sample diluent is added in to a dilution tube. Then vortexed to dilute the sample. The required number of Microwells on holder were placed. A1 left empty for Blanking. 100 µl of NC, 100 µl of Calibrator in duplicate were dispensed into respective wells. 100 µl of PC was dispensed in single. Then 100 µl of samples were dispensed in respective wells. All the samples are checked for blue color except the blank. Samples were incubated for 60 min at 37°C. The strips were covered. Wells were washed for 5 cycles. 100 µl of Ag/Ab Immunocomplex dispensed into each well except blank well. The strips were covered. All the wells are checked for red color except A1. Incubated for 60 min at 37°C and washed for 5 cycles. 100 µl of Chromogen/substrate mixture dispensed into each well including A1. Incubated for 20 min at 25°C. 100 µl of Sulphuric acid as stopping solution was

dispensed into all the wells. PC & Positive samples turned from blue to yellow. OD value measured at 450nm filter, 620-630 nm (backgroundsubstraction).

ISOLATION OF CELLS FROM THE SWAB

The swabs placed in 70% ethanol test tube is vortex mixed to loosen the cells in the swab and then the swab was discarded .The test tube containing 70% ethanol and the collected cells is centrifuged at 8000 rpm for 1 minute so that the cells get deposited in the bottom of the tube. The 70% ethanol is discarded leaving the cell deposits in the tube to which 0.5ml of PBS buffer is added. The cell deposits is transferred to a 1.5ml micro centrifuge tube for DNA isolation.

POLYMERASE CHAIN REACTION

ISOLATION OF VIRAL NUCLEIC ACID

PRINCIPLE:

Cells are lysed during a short incubation with chaotropic salt, which immediately inactivate all nucleases. Cellular nucleic acids bind selectively to special glass fibers pre-packed in the purification filter tubes. Bound nucleic acids are purified in a series of rapid ‘wash and spin’ steps to remove contaminating cellular components.

A special inhibitor removal buffer has been included which removes inhibitors from the preparation. Finally elution buffer releases the nucleic acids from the glass fiber. This simple method eliminates the need for the organic

solvent extractions and nucleic acid precipitation, allowing for rapid purification of many samples simultaneously.

MATERIALS REQUIRED

1. Micro pipette variable volume 0.5-10 , 10-100 and 100-1000
2. Sterile pipette tips with aerosol barrier 2-20, 10-100 and 100-1000
3. Disposable powder free gloves
4. Vortex mixer/ water bath
5. Centrifuge with rotor for 1.5 ml reaction tube
6. 1.5ml/ 2ml centrifuge tubes

PROCEDURE:

Before use all the reagents were thawed completely, mixed and centrifuged briefly**.**

1. The following reagents are added to a nuclease free 1.5ml centrifuge tube:
   a. 200µl of lysis buffer
   b. 5µl of carrier RNA was added
   c. 200µl of cell Deposit

d. 20µl of proteinase K

e. 5µl of internal template control

2. Centrifuge tube was mixed immediately by inverting it.

3. The tube is incubate at 56° C for 15min in the water bath.

4. After taking the tube from incubator 300µl of 100% ethanol was added and mixed by a vortex for 30 sec.

5. Then the tube was centrifuged for few seconds to bring down drops to bottom of the tube.

6. Entire sample was then transferred into the pure-fast spin column.

7. The pure fast spin column was centrifuged at 12000 rpm for 1 min.

8. The flow-through was discarded and column was placed back into the same collection tube.

9. To this, 500µl of 70% ethanol was added to the pure-fast spin column.

10. The pure fast spin column was centrifuged at 12000 rpm for 1 min and the flow-through was discarded and column was placed back into the same collection tube.

11. The empty spin column was attached with collection tube and centrifuged at 12000rpm for an additional 2 min (essential to avoid residual ethanol) and the collection tube was discarded.

12. The pure fast spin column was then transferred to a fresh 1.5ml micro centrifuge tube, to this, 50µl of elution buffer was added to the Centre of pure fast spin column membrane and incubate for 2 min at room temperature.

13. The pure fast spin column and the centrifuge tube was centrifuged at 12000rpm for 1 min and the pure fast spin column was then discarded.

14. Now the centrifuge tube containing the eluted nucleic acid was stored at -80°C for later analysis.

Preparation of agarose gel

2% agarose gel was prepared by adding two grams of agarose powder to 100 ml of electrophoresis buffer, then heated in a microwave oven for 3 min, mixed until the agarose was evenly dissolved. After cooling to about 60°C.5 µl of ethidium bromide was added to 100 ml of the gel to enable visualization of DNA after electrophoresis. Ethidium bromide being carcinogen being handled with precaution.

A well-formed COMB/template was placed across the end of the casting tray which is covered with a cellophane tape in its both the ends and the freshly prepared gel was poured into the casting tray which act as a mold. This was let to solidify at room temperature.

Preparation of Electrophoretic buffer

Electrophoresis (TAE- Tris Acetic acid –EDTA) buffer was prepared in the 50x dilution. To prepare a 1000ml of buffer 980ml distilled water and 20ml of buffer is added and poured into the electrophoresis tank. After the gel has hardened enough, the gel was gently placed on the electrophoresis tank with buffer and was made sure that the gel was completely immersed in the buffer and comb was removed carefully from the gel.

SEMI NESTED POLYMERASE CHAIN REACTION FOR HERPES SIMPLEX VIRUS 2

MASTER MIX

Master mix is prepared taking 10µl of master mix and 10µl HSV2 primer mix for one sample and mixed together.20µl of this mixture is added to a 0.2ml PCR tubes to which 5µl of sample is added. Positive control and negative control are also added in the similar method.

The primer sequence of the HSV2 used in this procedure is

5'-GCGTTTCTGATCGACGACGCCT-3'

5'-TTTTAACCGCCGCCAGCACGTT-3

Thermocycling

The 0.2ml tubes having the positive, negative controls and the sample are placed in the thermo cycler and the program has been set to run in the following steps.

1. Denaturation at 95^{o}C for 30 seconds
2. Annealing at 54^{o}C for 30 seconds
3. Primer extension at 72^{o}C for 30seconds
4. Again step 1.the cycle is repeated for 35 times

Gel electrophoresis

After the thermocycler finished the amplification cycles (35 cycles) the tubes are taken out from the thermocycler and then 5µl of DNA ladder, 20 µl sample, 20 µl negative control and positive controls and were loaded into the gel wells using micropipette. Electrical leads were connected to the electrophoresis tank. And the current was supplied with a voltage 150V so that negatively charged DNA migrate from cathode to anode. Gel running time was approximately the time taken by the gel loading dye to cover three fourth of distance in the gel.

After the gel electrophoresis, PCR products were observed using UV Transilluminator. The DNA ladder is a mixture of fragments with known size to

compare with the PCR fragments.Amplicons of size 111bp of glycoprotein G gene(gG) were consistent with viral DNA.

MULTIPLEX POLYMERASE CHAIN REACTION FOR HUMAN PAPILLOMA VIRUS 16 AND 18

MASTER MIX

Master mix is prepared taking 10µl of master mix and 10µl HPV16 &18 primer mix for one sample and mixed together.20µl of this mixture is added to a 0.2ml PCR tubes to which 5µl of sample is added. Positive control and negative control are also added in the similar method.

The primer sequence of HPV16 & 18 used in this procedure are

HPV16

5'-GCAACAGTTACTGCGACGTGAGGT-3’

5'-CACACAACGGTTTGTTGTATTGCTGT-3'

HPV18

5'-TGATCTGTGCACGGAACTGAACAC-3'

5'-TCAACGGTTTCTGGCACCGCAG-3'

Thermocycling

The 0.2ml tubes having the positive, negative controls and the sample are placed in the thermo cycler and the program has been set to run in the following steps.

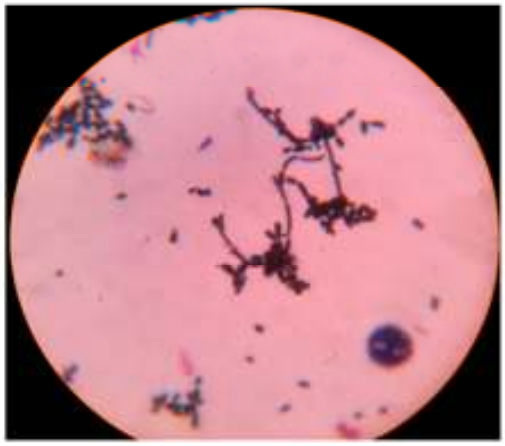
1. Denaturation at 95^{o}C for 30 seconds
2. Annealing at 54^{o}C for 30 seconds
3. Primer extension at 72^{o}C for 30seconds
4. Again step 1.the cycle is repeated for 35 times

Gel electrophoresis

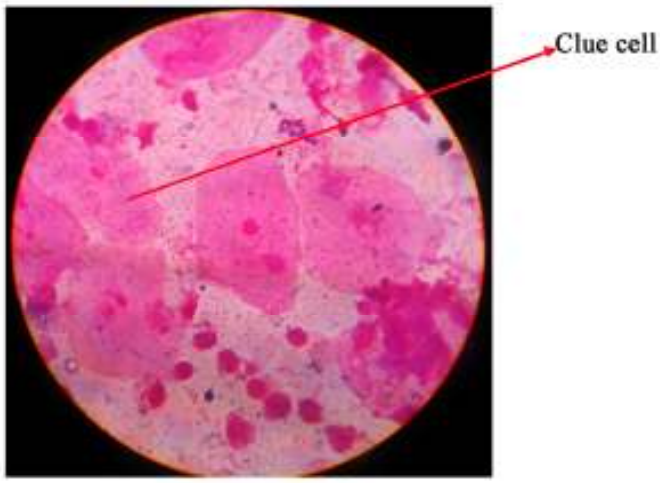
After the thermo cycler finished the amplification cycles (35 cycles) the tubes are taken out from the thermo cycler and then 5µl of DNA ladder, 20 µl sample, 20 µl negative control and positive controls and were loaded into the gel wells using micropipette. Electrical leads were connected to the electrophoresis tank. And the current was supplied with a voltage 150V so that negatively charged DNA migrate from cathode to anode. Gel running time was approximately the time taken by the gel loading dye to cover three fourth of distance in the gel.

After the gel electrophoresis, PCR products were observed using UV Transilluminator. The DNA ladder is a mixture of fragments with known size to compare with the PCR fragments.Amplicons of size 200bp and 300bp were consistent with HPV 16 E7 and 18 E7respectively were taken as positive

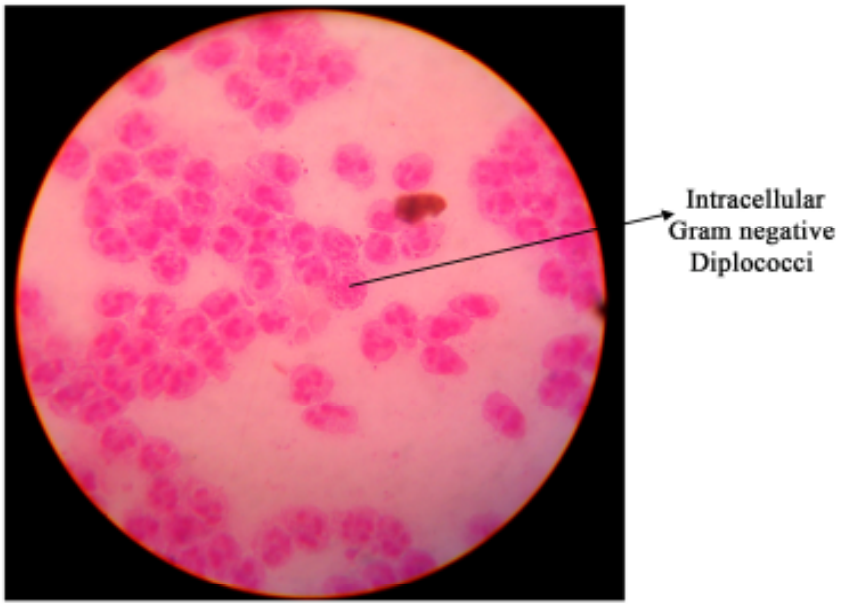
Budding yeast cells with Pseudo hyphae



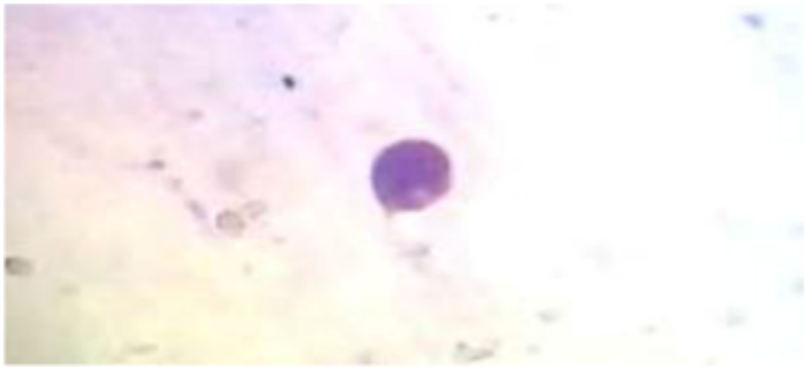
Bacterial vaginosis - Clue cells



Direct gram stained rectal smear showing pus cells with Intra cellular Gram negative Diplococci

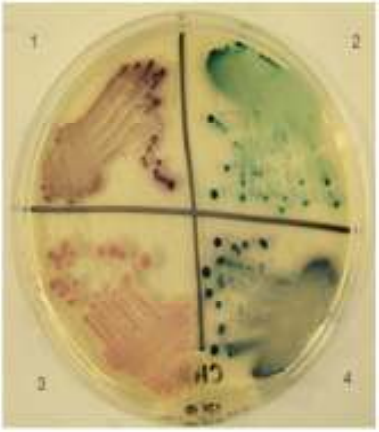


Giemsa stained culture smear of Trichomonas vaginalis :



CPLM Medium for
Trichomonas vaginalis

Candida Chrom Agar

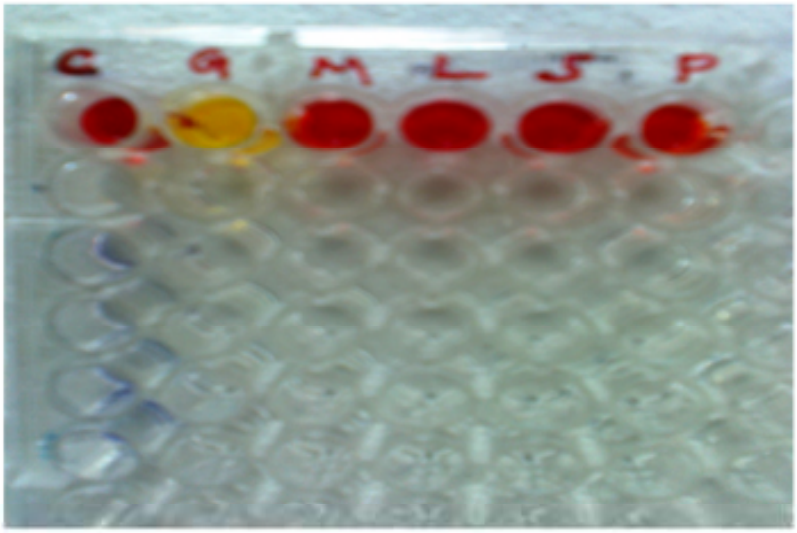


CHROMagar: 1=C glabrata;
2= C albicans 3=C krusei;
4=C tropicalis

Sub culture of Neisseria gonorrhoeae in MTM agar plate

Antimicrobial susceptibility testing for Neisseria gonorrhoeae

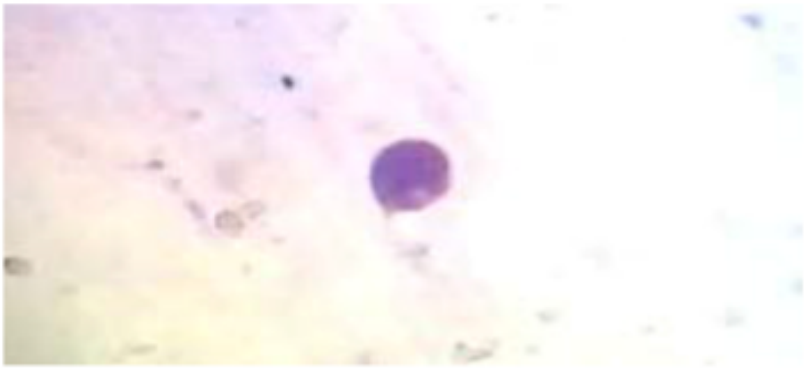
RAPID CARBOHYDRATE UTILIZATION TEST



Before incubation

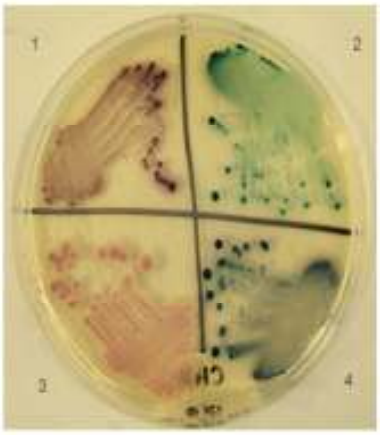
After incubation

Giemsa stained culture smear of Trichomonas vaginalis :



CPLM Medium for
Trichomonas vaginalis

Candida Chrom Agar

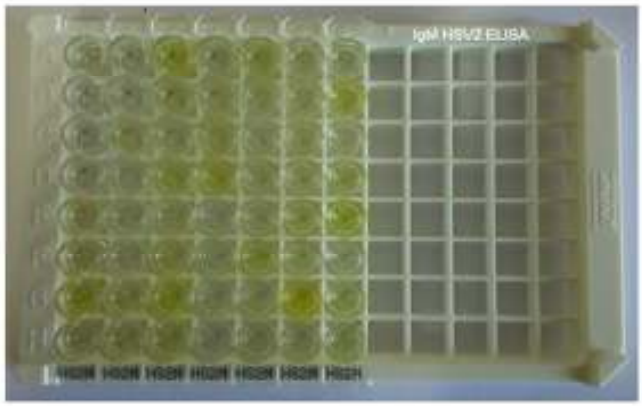


CHROMagar: 1=C glabrata;
2= C albicans 3=C krusei;
4=C tropicalis

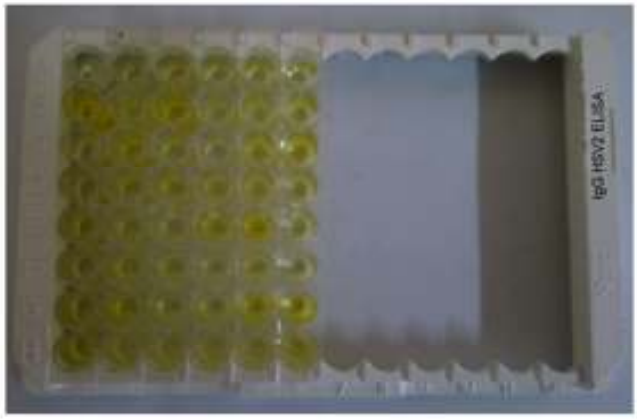
Rapid card test for Hepatitis C virus

Rapid card test for Hepatitis B virus

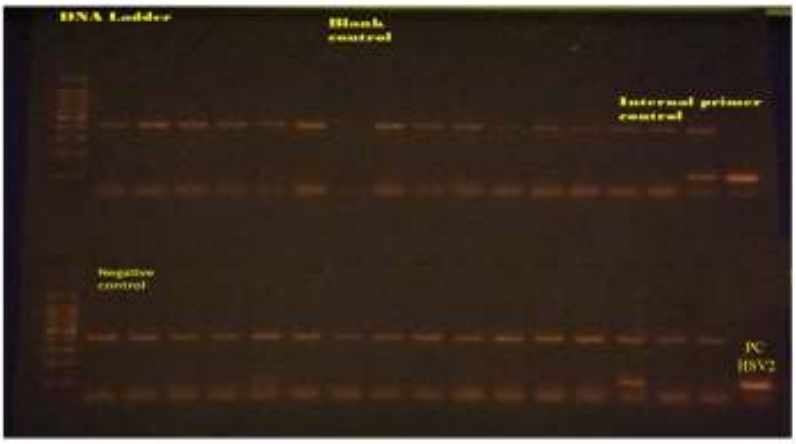
Results of IgM Sandwich ELISA for HSV2



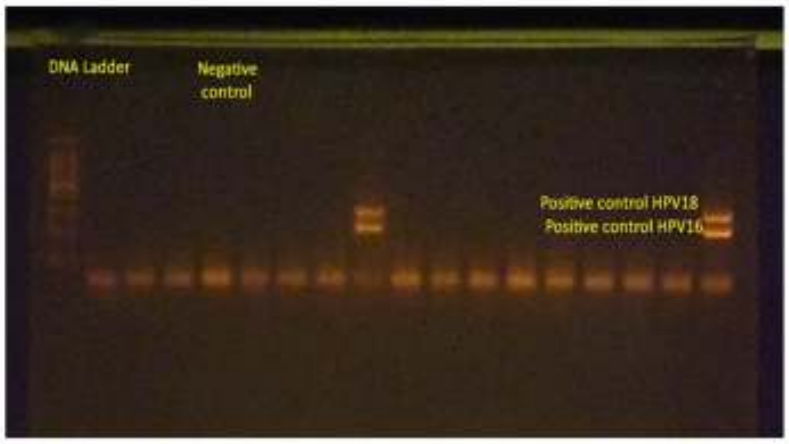
Results of IgG Sandwich ELISA for HSV2



Semi nested PCR for Herpes Simplex Virus 2



Multiplex PCR for Human Papilloma Virus 16 and 18



OBSERVATION AND RESULTS

OBSERVATION & RESULTS

Two hundred patients (female Sex Workers-114, Males who have Sex with Males-60, Transgenders-26) who were asymptomaticwererecruited and included in this studyfrom STD clinic. Specimens such as High Vaginal, Endocervical, Pharyngeal, rectal swabs and blood were collected and processed to detect etiological organismsat the Department of Microbiology. The results were analyzed as follows.

Table-1: Age Distribution

Age in years	Males who have Sex with Males(MSM)	Female Sex Workers(CSW)	Trans genders(TG)	Total
18-20	3(5%)	1(0.8%)	0	4(2%)
21-30	33(55%)	47(41.2)	18(69.2%)	98(49%)
31-40	17(28.3%)	54(47.3%)	7(27%)	78(39%)
41-50	7(11.4%)	12(10.5)	1(3.8%)	20(10%)
Total	60	114	26	200(100%)
P value	0.128			

The commonest age group among MSM is 21-30(55%).Among the female Sex Workers (FSW) the commonest age group is 31-40(47.3%).In transgenders (TG)the commonest age group is21-30(69.2%) and the overall commonest age group in the high risk group is 21-30(49%).

1. Age Distribution

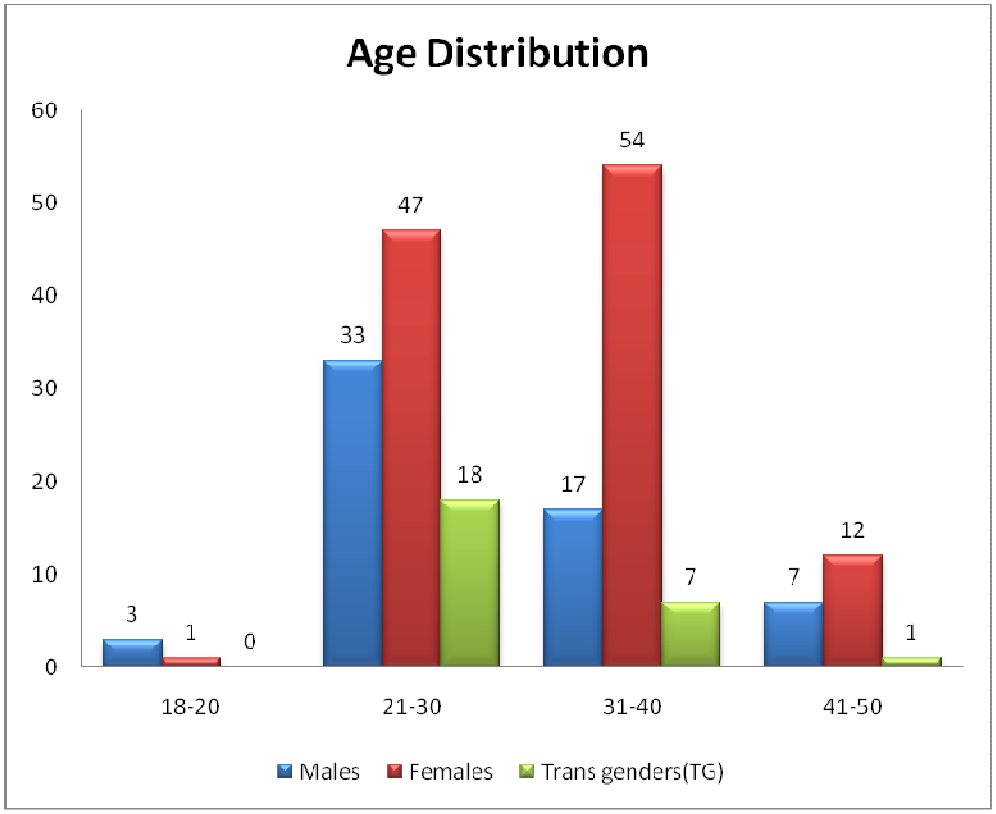


Table 2: Marital status

Category	Married	Un married	Separated	Divorced	Total
Female Sex Workers	48(42.2%)	40(35%)	24(21%)	2(1.8%)	114
Males who have Sex with Males	20(33.3%)	40(66.7%)	0	0	60
Trans genders	0	26(100%)	0	0	26
Total	68(34%)	106(53%)	24(12%)	2(1%)	200

Marital status among the high risk groups is that most of them are unmarried 106(53%).

2. High Risk Categories

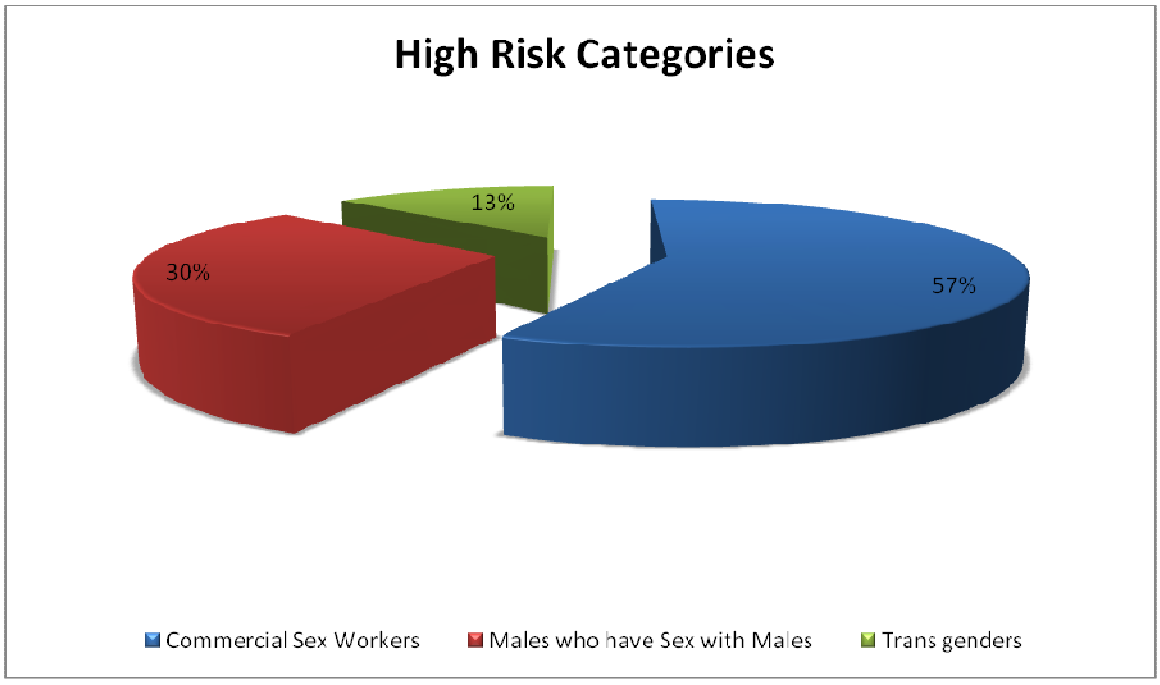


Table-3: Sexual History

TYPE	Female Sex Workers	Males who have Sex with Males	Trans genders	Total	P value
Heterosexualmode	114	0	0	114(57%)	P < 0.01
Homosexual mode	0	40	26	66(33%)	P < 0.05
Bisexual mode	0	20	0	20(10%)	P < 0.01

Among the high risk groups the commonest mode of sex is heterosexual mode 134(57%).

3.Sexual History

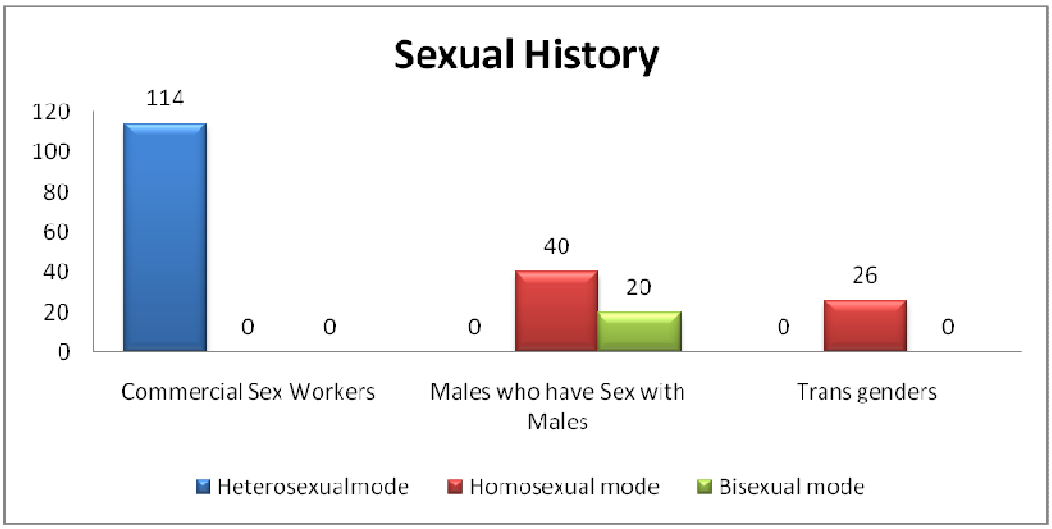


Table-4: Mode of sex among homosexuals

TYPE		Males who have Sex with Males	Trans genders	Total	
Kothis	Oro Receptive	1(1.6%)	3(11.5%)	4	29 (33.7)
	Ano Receptive	0	0	0	
	Ano/oro Receptive	2(3.4%)	23(88.5%)	25	
Panthis	Ano Insertive	21(35%)	0	21	42 (48.8%)
	Oro Insertive	7(11.6%)	0	7	
	Ano/oro Insertive	14(23.4%)	0	14	
Double deckers	Ano/oro Insertive &oro receptive	15(25%)	0	15	15 (17.5%)
Total		60	26	86	86
Df		20			
P value		0.001			

Male sex workers (MSWs) are classified into different categories based on identity, Gender, behavior, and profession. Based on identity they are categorized as “gay,” “bisexuals,” “Kothis,” (ano/oro, both receptive partner) or “Panthis” (ano/oro both insertive partners) and double decker (both ano/oro receptive and insertive) Among the MSMs the commonest group is Ano/oro Insertive (panthis) (48.4%) and the transgenders indulge in ano/oro receptive method 23 (88.5%).

Mode of sex among Homosexuals

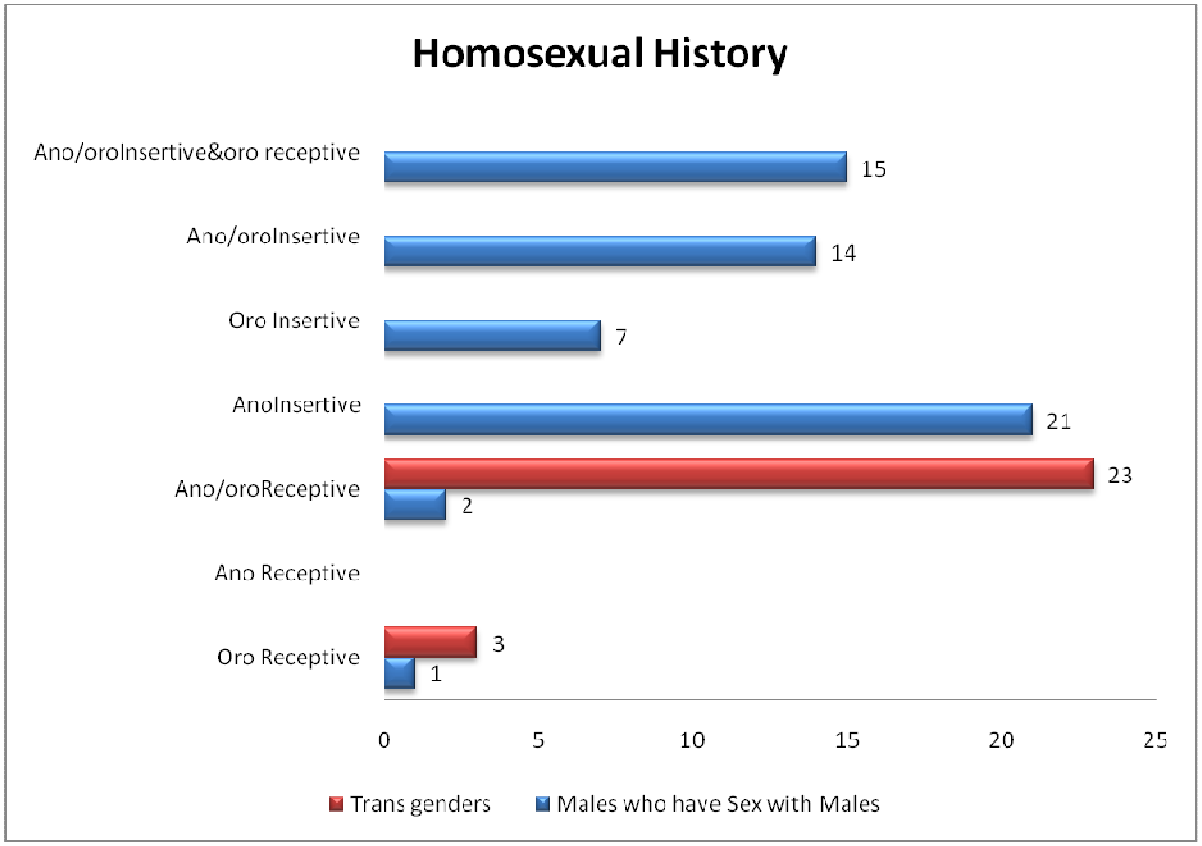


Table-5: Protective Measures & Blood Transfusion History

Category	Unprotected	Protected	H/O Blood Transfusion
Female Sex Workers	43	71	1
Males who have Sex with Males	23	37	1
Trans genders	16	10	0
Total	82(41%)	118(59%)	2(1%)

Protected last sexual contact was seen in 118(59%) and persons with the history of blood transfusion is 2 (1%)

Protective Measures & Blood Transfusion History

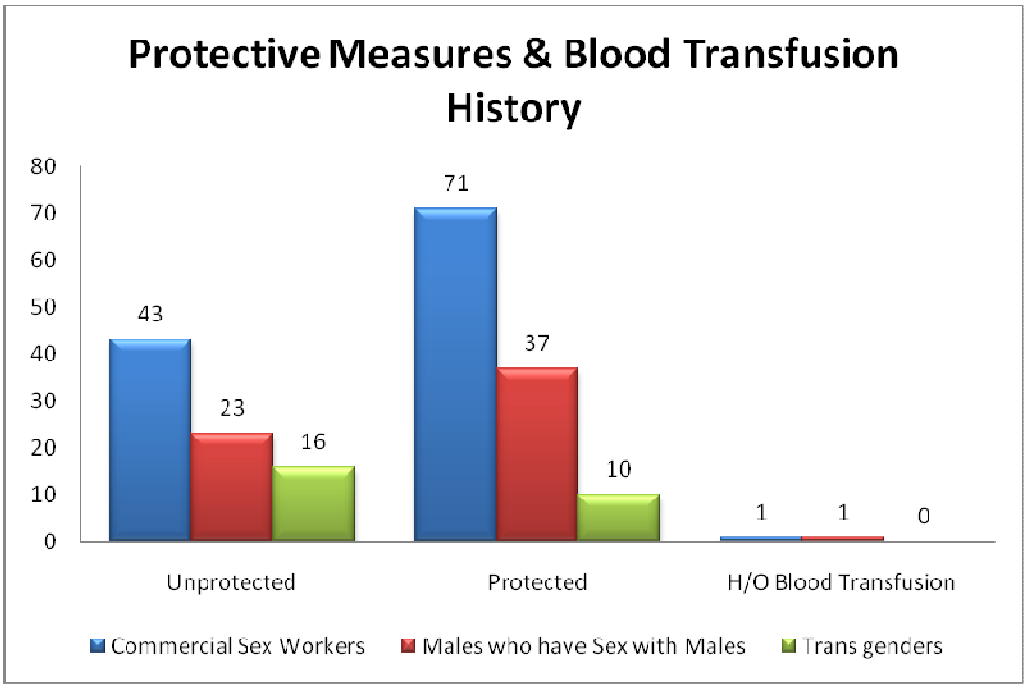


Table -6: Sex Reassignment Surgery (SRS)

Category	SRS done	SRS not done
Trans genders	18	8
Total	18	8
Percentage	69.2%	30.8%

Among the transgenders the number of persons undergone Sex Reassignment Surgery (SRS) was 18(69.2%)

Sex Reassignment Surgery (SRS)

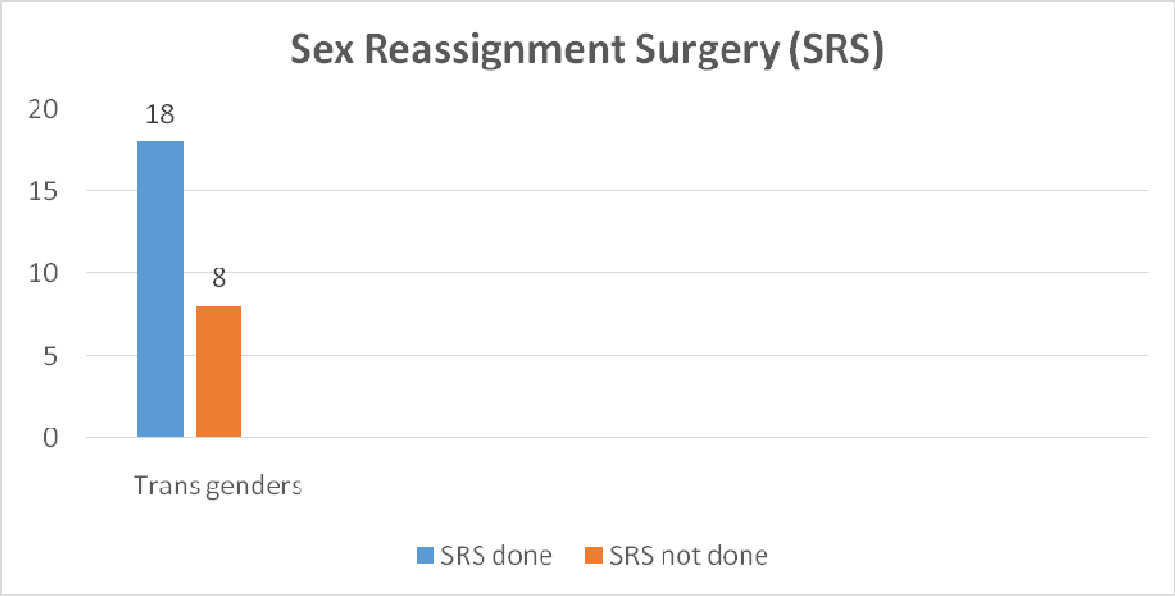


Table-7: Nature of specimens among High risk group

Category	Pharyngeal Swab	Rectal Swab	Endocervical Swab	High Vaginal Swab	Urethral swabs	Blood
female Sex Workers	23	00	114	114	0	114
Males who have Sex with Males	60	24	00	00	60	60
Trans genders	26	26	00	00	0	26
Total	109	49	114	114	60	200

Among the high risk groups endocervical, high vaginal swab was collected 114patients. In the Males who have Sex with Males pharyngeal swabs was collected predominantly (60) and rectal swab in 24.In the transgenders pharyngeal and rectal swab was collected for all of them (24).The blood was collected from all the group (200).

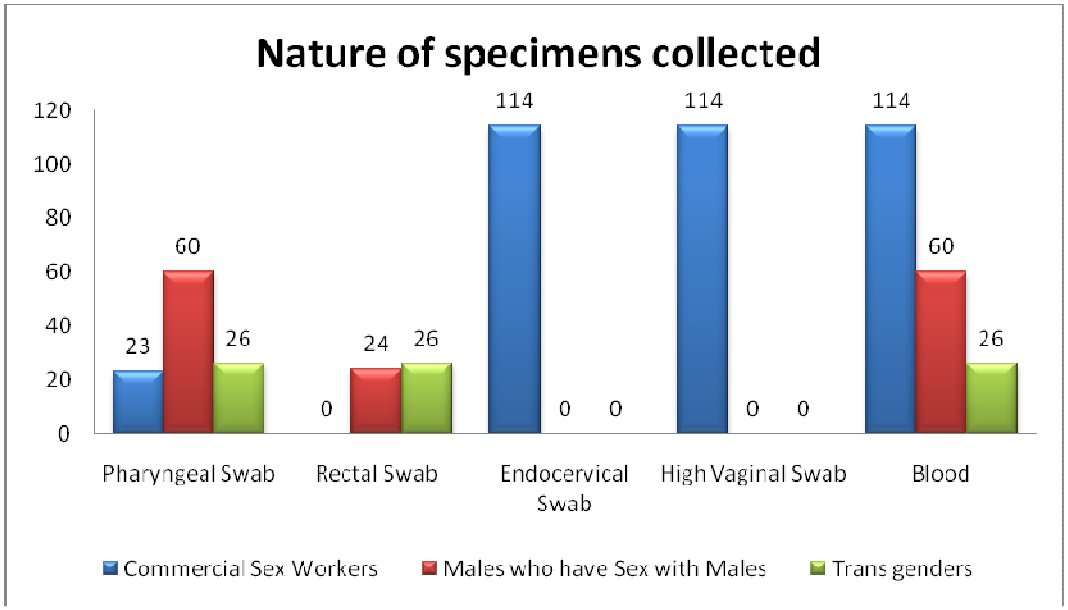


Table 8: Positivity in Direct examinations

Tests done	Positive findings		High Vaginal Swab (114)	Pharyngeal Swab (109)	Rectal Swab (49)	Endocervical Swab (114)	Urethral swabs (60)	Total
Wet mount (114)	Clue cells		6(5.2%)	-	-	-	-	6
	Yeast cells with/without budding mycelium		17 (14.9%)	1	-	-	-	18
	Motile Trichomonads		1	-	-	-	-	1
10% KOH mount (114)	Yeast cells with/without budding mycelium		17	1	-	-	-	18
	Fishy odour/Amine test		6	-	-	-	-	6
Gram Stain (446)	Epithelial cells with lactobacillus		110	-	-	-	-	110
	Clue cells		6	-	-	-	-	6
	Nugent score (7-10)	Lactobacillus 1-4,<1	4	-	-	-	-	4
		Mobiluncus >5,1-4	4					
		Gardernerella 5-30,>30	4					
	Pus cells & Intra cellular Gram negative diplococci			-	1	-	-	1
	Yeast cells with/without budding mycelium		17	1	-	-	-	18

Among the high vaginal swabs (114) collected in female Sex workers, the wet mount examination showed clue cells in 6(5.2%) which is in accordance with gram staining, Yeast cells with/without budding mycelium was seen in 17(14.9%) which is in accordance with 10% KOH mount and motile trichomonads was seen in 1(0.5%).Gram staining of the specimens collected showed Pus cells & Intra cellular Gram negative diplococci in 1(2%) in rectal swab and although the gram staining showed clue cells in 6(5.2%) .Nugent's score of 7 to 10 was seen in only 4(3.5%).

Table-9: Culture positivity

Culture Done	Result	Female Sex Workers (114)	Males who have Sex with Males (60)	Trans genders (26)	Total (200)
Modified Thayer martin medium(200)	Positive	0	0	1(Rectal)	1(0.5%)
Human Blood Tween 80 Bilayer Medium(114)	Positive	4	0	0	4(3.5%)
CPLM medium(200)	Positive	1	0	0	1
SDA agar(200)	Positive	17	0	1	18(9%)
CHROMagar agar(200)	Positive	17	0	1	18(9%)

Among the high risk groups the culture for Gonococci, Modified Thayer martin medium was positive in 1(0.5%) from the rectal swab of the Transgender. The culture for bacterial vaginosis Human Blood Tween 80 Bilayer Medium was positive in 4(3.5%) in female sex workers. The culture for Trichomoniasis, the CPLM medium was positive in 1(0.5%) in a female sex workers. The culture for candidiasis, SDA culture was positive in 18(9%).

Table-10: Agreement between Direct examination and Culture

Culture /direct smear		Female Sex Workers	Males who have Sex with Males	Trans genders	Total
Gonorrhoeae	Direct Gram Stain positive	0	0	1*	1
	Culture Positive	0	0	1*	1
Bacterial Vaginosis	Direct Gram Stain positive	6	0	0	6
	Culture Positive	4	0	0	4
Trichomoniasis	Direct wet mount positive	1	0	0	1
	Culture Positive	1	0	0	1
Candidiasis	Direct Gram Stain positive	17	0	1	18
	Culture Positive	17	0	1	18

*---Rectal

There was 100% agreement between gram stain and culture of the Gonococci (1) and 100% agreement between gram stain and culture of the candidiasis (18) and also 100% agreement between wet mount and culture of the Trichomoniasis(1) whereas in the Bacterial vaginosis culture it is 4 out of 6 (66.6%)

Table-11: Antimicrobial Susceptibility Testing for*Neisseria gonorrhoeae*

Disk	content	No of isolates 1(Rectal)		
		Resistant	**Intermediate**	**Susceptible**
Penicillin	10 IU	R*		
Ciprofloxacin	5µg	R		
Azithromycin	15 µg	R		
Tetracycline	30 µg		I*	
Ceftriaxone	30 µg		I	
Spectinomycin	100 µg			S*

R-Resistant S-sensitive I -intermediate

Isolate was resistant to Penicillin andsensitive to Spectinomycin.

Table -12 Candida Speciation

Candida Species	Female Sex Workers	Males who have Sex with Males	Trans genders	Total
Candida albicans	9	0	0	9(50%)
Candida krusei	4	0	1	5(27.8%)
Candida glabrata	2	0	0	2(11.1%)
Candida parapsilosis	2	0	0	2(11.1%)
Candida tropicalis	0	0	0	0
Total	17	0	1	18

Candida albicans was 9(50%) and 50% of candida non albicans of which candida krusei 5(27.8%) is predominant.

Table-13: Positivity in Serological tests

Test Done		Female Sex Workers 114	Males who have Sex with Males 60	Trans genders	Total
HIV test	Known reactive on ART	2(1%)	2(1%)	4(2%)	8(4%)
	reactive	0	0	0	0(0%)
RPR	Reactive	3(1.5%)	3(1.5%)	1(0.5%)	7(3.5%)
TPHA	Positive	3(1.5%)	3(1.5%)	1(0.5%)	7(3.5%)
HBsAg rapid card	Positive	1(0.5%)	0	0	1(0.5%)
Hep C rapid card	Positive	0	0	0	0(0%)
ELISA IgM HSV2	Positive	8(4%)	5(2.5%)	3(1.5%)	16(8%)
ELISA IgG HSV2	Positive	37(18.5)	19(9.5%)	11(5.5%)	67(33.5%)

In the serological tests the known HIV patients on ART were 8(4%) and the RPR/TPHA positives were 7(3.5%) and the Hepatitis Surface antigen was seen in 1(0.5%).The ELISA for IgM HSV2 antibody was seen in 16(8%) and the IgG HSV2 antibody was seen 67(33.5%).

Table-14Results of Combined IgM &IgG HSV 2 capture ELISA

Categories	IgM Positive	IgG Positive	Both IgM & IgG Positive
female Sex Workers	8	37	8
Males who have Sex with Males	5	19	5
Trans genders	3	11	3
Total	16(8%)	67(33.5%)	16(8%)

IgM HSV2 was positive in 16(8%) and the IgG was positive in 67(33.5%) and the patients with both IgM and IgG are 16(8%).All the IgM positive patients are also IgGpositive.

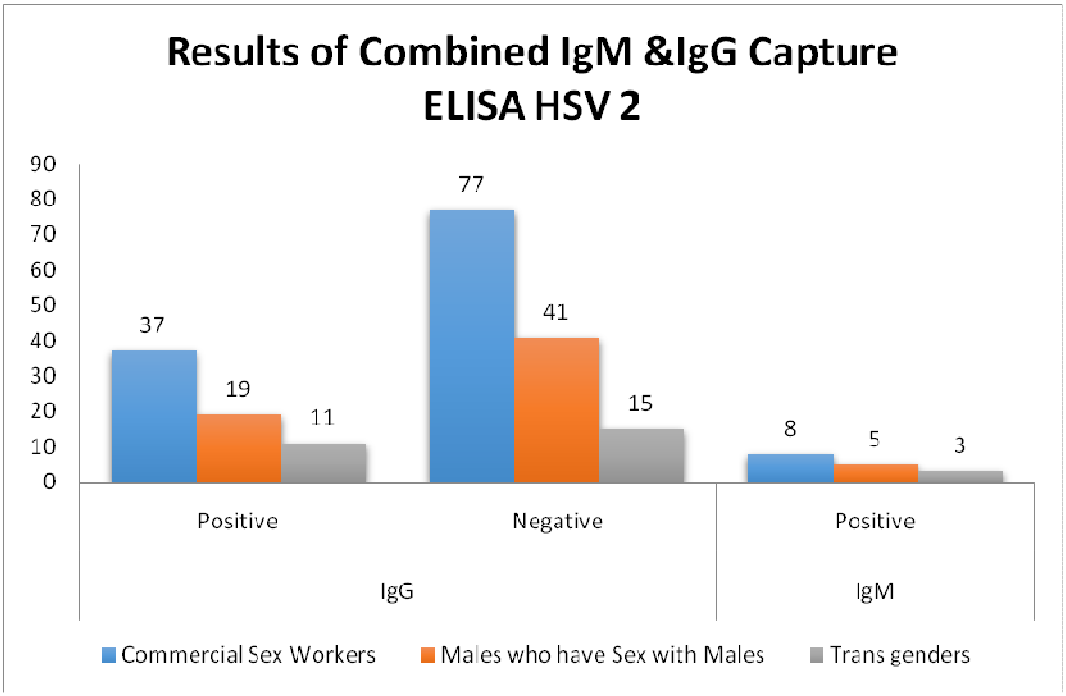


Table-15: PCR for HSV2 from Anogenital swabs

Results	Female Sex Workers	Males who have Sex with Males	Trans genders	Total
Positive	10(5%)	6(3%)	4(2%)	20(10%)
Negative	104	54	22	180(90%)
Total	114	60	26	200
Df	2			
Pearson Chi-Square	0.784			

The semi nested PCR for HSV2 showed positive in 20(10%) of which most of them are female sex workers 10(5%)

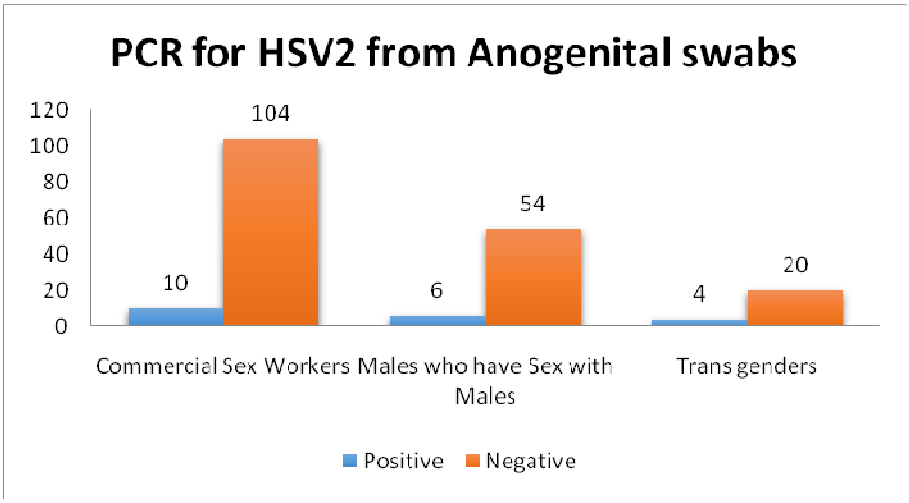


Table-16

Comparison of PCR HSV2 Positive cases and Combined IgM & IgGCapture ELISA HSV 2

Categories	PCR HSV2Positive	IgMPositive	IgGPositive
female Sex Workers	10	8	37
Males who have Sex with Males	6	5	19
Trans genders	4	3	11
Total	20(10%)	16(8%)	67(33.5%)

Among the 20 positive HSV2 PCRonly 16 cases were positive for IgM &IgG and all the PCR positive cases were IgG positive.

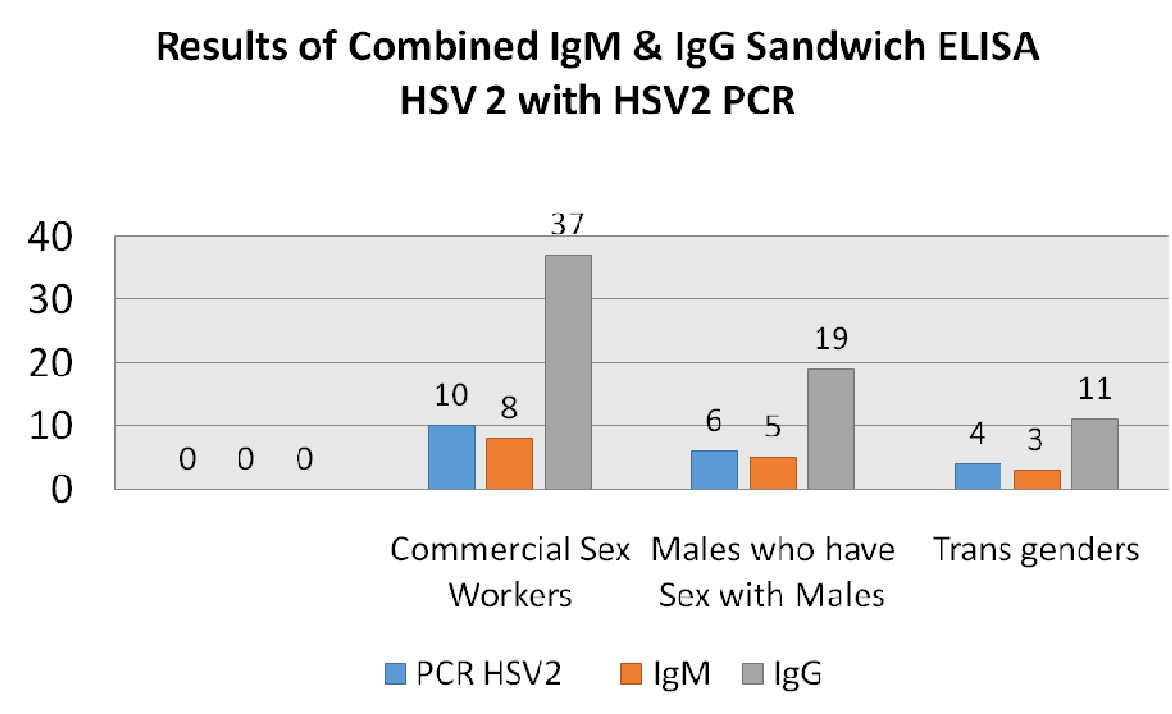


Table-17: PCR for HPV 16 & 18 from Anogenital swabs

Results	Female Sex Workers	Males who have Sex with Males	Trans genders	Total
HPV 16 Positive	2	0	0	2(1%)
HPV 18 Positive	1	0	0	1(0.5%)
HPV 16 & 18 Positive	1	0	0	1(0.5%)
Total	4	0	0	4(2%)

HPV 16 was positive in 2 cases, HPV 18 positive in one and HPV 16 and 18 co infection in 1 (0.5%).

Table-18: Co-infection

Co-infection	Frequency			
	Female Sex Workers	Males who have Sex with Males	Trans genders	Total
Syphilis &HIV	0	0	1	1
HSV 2&HIV	2	2	2	6
HPV 16 & 18and HIV	1	0	0	1
Candidiasis &HIV	0	0	1	1

Among the high risk groups the co infection of Syphilis withHIV is 1(14.2%), co infection of HSV2 with HIV is 6(8.9%), co infection of HPV 16& 18 withHIV is 1(25%) and the co infection of Candidiasis with HIV is 1(5.5%).

DISCUSSION

More than 1 million people acquire a sexually transmitted infection every day. It is estimated that about 500 million people acquire one of the four following sexually transmitted infections Chlamydia, Gonorrhoea, Syphilis and Trichomoniasis every year.The incidence of sexually transmitted viral infections has increased in the recent past which is due to the increased awareness of people seeking healthcare facilities, increased use of broad spectrum antibiotics and the effectiveness of syndromic approach of treatment[2].

More than 530 million people are living with HSV2 and more than 290 million women have an HPV infection which is one of the most common STIs[30].The risk of acquiring the STIs and spreading it to the community is more in the high risk groups because of the promiscuous sexual activity, unprotected sex and the young age group making them more vulnerable.

A total of 200 high risk group patients were included in this study who were all asymptomatic at the time of study. Demographic information about the high risk groups like age group, marital status, sexual behavior were collected and appropriate samples were collected. Standard laboratory procedures were used for collection, transport and processing the samples.

Among the high risk groups taken for study the female sex workers (57%) were the commonest among them compared to males having sex with males (30%) and transgenders (13%).The commonest age group among the high risk group is the 21-30(49%) since people will be more sexually active in this

age group and this is similar with the study by S Abarnadevi et al[31] in which the commonest age group is 21-30(39%).

In our study the marital status among the high risk groups is that most of them are unmarried 106(53%), married 68(34%) separated and divorced (13%). InBach et al study[33] the prevalence of marital status among the high risk groups as unmarried (46.8%), married (14.2%) and separated and divorced (39%).

In our study the predominant mode of sexual contact was heterosexual mode (57%), followed by homosexual mode (33%) and bisexual mode in 10%.S Abarnadevi et al[31]study showed heterosexual mode (89.5%) homosexual mode (1.6%) and bisexual (4.6%) of patients. Heterosexual mode was predominant because of the fact that female sex workers had multiple sexual partners.

Male sex workers (MSWs) are classified into different categories based on identity, Gender, behavior, and profession. Based on identity they are categorized as “gay,” “bisexuals,” “Kothis,” (ano/oro, both receptive partner) or “Panthis” (ano/oro both insertive partners) and double decker (both ano/oro receptive and insertive).In our study as per the identity there are 29(33.7%) Kothis and 42(48.8%) panthis and 15(17.5%) double decker. InBrahmam et al[50] study it was shownto be Kothi (49.9%), panthi (7.7%) and double decker (28.5%).Among the MSMs the commonest group is Ano/oro Insertive (panthis) (48.4%) and in transgenders it is ano/oro receptive method 23 (88.5%).

In our study a history of protected last sexual contact (with condoms) was elicited in 59% and 41% had unprotected sexual act (without condoms) among the high risk groups patients which correlates with the study of S Wee et al[32] where 55% used condoms during sex.Eventhough awareness about condom usage is present they cannot adhere to it due to - Impaired decision making about condom usage when they are under the influence of alcohol, inaccessibility of condom at the time of need and misbelief that using condoms will reduce the pleasure during the act.

Among the high risk groups in the female sex workers who were willing for genital examination the endocervical, high vaginal swab and blood was collected he pharyngeal swab was collected only for 23 because the female sex workers say they seldom indulge in oral sex and were not willing for pharyngeal swab .In the Males who have Sex with Males genital and pharyngeal swab was collected for ano/oro receptive and insertive groups and rectal swab is collected in 24,who had ano and oro receptive sex.

In our study the analysis of direct examination of clinical specimen results were out of 223 wet mount done 6 showed clue cells (2.69%), 18 showed yeast cells with/without budding mycelium (8.07%) and motile trichomonads was 1(0.4%). Of 223 KOH mount 18 were positive for Yeast cells with/without budding mycelium (8.07) and among the 446 gram staining done 6 were positive for clue cells (1.35%) and 18 were positive for Yeast cells with/without

budding mycelium(4.03%) and one was positive for Pus cells & Intra cellular Gram negative diplococci(0.2%)

Among the high risk groups the direct examination of the samples collected were in the pharyngeal swabs (109) wet mount positive for yeast cells with budding mycelium was one (0.9%) transgender which correlated with the gram staining. In the rectal swabs (49) collected, gram staining showed one sample (2%) positive for pus cells and Intra cellular Gram negative diplococci in a transgender. In the vaginal swabs collected the wet mount showed 6(5.2%) clue cells which correlated with the gram staining and the yeast cells with budding mycelium was seen in 17(14.9%) which correlated with the gram staining and the other vaginal smear showed normal epithelial cells with lactobacillus.

Tables11, 12 and 13

Among the high risk groups the Modified Thayer martin medium culture positivity is one (0.5%) from the rectal swab of an asymptomatic transgender in whom the gram staining showed pus cells along with gram negative diplococci which is low when compared with Beena Thomas et al[41] in which the prevalence is 4.7%.

Among the female sex workers in our study the presence of clue cells in gram staining and wet mount was 6(5.2%) but the culture positivity and the Nugent's score of 7 to 10 is 4(3.5%) .The correlation between culture method

and gram staining was 4 out of 6(66.6).In Spiegel et al[49] the correlation was 49 out of 60(81.6%).The prevalence of Bacterial vaginosis among the asymptomatic female sex workers is 3.5% whereas in the study conducted by Wiset et al[42]the prevalence is 24-37% among the asymptomatic women.

In our study the prevalence of Trichomoniasis among the high risk group is 1(0.5%) to which the wet mount also positive for motile trichomonads. Study by Nimisha D shethwala et al[43]showed prevalence of Trichomoniasis is 2%.

In our study the correlation between culture method and gram staining for candidiasis was 100% which correlates with Omar AA et el[51] in which the correlation was 94.9%. In our study the prevalence of candidiasis among the high risk groups is 18(9%) which is similar with the study of Nimisha D shethwala et al[43] in which the prevalence is 10.33%.

Candida speciation was done which revealed that candida albicans 9(50%) and non albicans 9(50%) of which 5(27.8%) is candida krusei, 2(11.1%) is candida glabrata and candida parapsilosis is 2(11.1%) which correlates with the study by Luciene et al[44] in which the prevalence candida albicans is 55.6% and non albicans prevalence is 44.4%..Earlier candida albicans was the predominant cause of candidiasis but now there has been increase in the prevalence of candida non albicans as the cause of candidiasis .This change in the etiological pattern should be taken in to consideration

because of the fact that non albicans are having increased resistance to the azoles which are used to treat the candidiasis

Serological analysis of our study showed HIVprevalence in the female sex workers (1%), in MSMs (1%) and in transgenders (2%) which was low when compared to recent NACO[34] statement that among men who have sex with men (MSM) and female sex workers (FSWs) it was 4.43% and 2.67% respectively. According to UNDP [35] among transgenders (TG) there is a very high HIV prevalence (17.5% to 41%) among them. In our study all the HIV infected patients are positive for IgG antibody against HSV2.

Rapid Plasma Reagin test and Treponema pallidum haemaggluttinin test was reactive for 3(1.5%) in female sex workers and 1(0.5%) in transgenders which is low when compared with UNDP [35] in which the prevalence of syphilis in transgenders is 13.6%.and the prevalence of syphilis in female sex workers is 8.2% as per Uribe-Salas get al[36] and the prevalence of syphilis among the men who have sex with men (MSM) is 3(1.5%) which is similar to the study by Pisani et al [37] in which the prevalence is 2%.

Hepatitis B among the female sex workers is 1(0.5%) which is low when compared with Todd CS et al[38] in which the prevalence is 6.54%.

Herpes simplex virus antibody IgM was seen in 16(8%) and the IgG antibody against HSV2 was 67(33.5%) estimated by Sandwich ELISA method. The prevalence of HSV2 in transgenders was 5.5% which is low when

compared with UNDP [35] in which the prevalence is 29%.Prevalence of HSV2 in female sex workers is 18.5% which correlates with S Navadeh et al[39] in which the prevalence is 18%.

The semi nested PCR for HSV2 DNA from the anogenital swabs were shown to be positive in 20(10%) which correlates with the Elizabeth Tronstein et al[40] in which the asymptomatic shedding of HSV2 DNA is 8.5%.Among the 20 positive samples 10 are from endocervical swabs of female sex workers, 6 penile swabs from men who have sex with men and 4 are from transgenders of which 3 are from the anal swabs and 1 from the pharyngeal swab.

All the 20 PCR positive samples was IgG antibody positive whereas the IgM is positive in only 16(8%) cases which implies the evidence of viral shedding even in the absence of IgM antibodies. Thus the interpretation of serological antibodies results reveals only the tip of the iceberg. These facts warrant us to equip the molecular methods to know about the genital viral shedding in both symptomatic and asymptomatic patients.

As per the study of Peter Leone et al[9]transmission of genital herpes frequently occurs during asymptomatic viral shedding. Famciclovir 250 mg bid has effectively decreased the incidence of both clinical and subclinical genital HSV shedding as per Peter Leone et al[9].Adopting such a prophylactic strategy in the high risk group will help to reduce the spread of genital herpes infection and also reducing the risk of acquiring the HIV among themselves.

In our study the prevalence of Human Papilloma virus 16 and 18(High risk types) by the multiplex PCR was 4(2%) of which 2(1%) was HPV 16 infected, one (0.5%) was coinfected with both HPV 16 and 18.It is low when compared Ishita Ghoshet al[29] in which the prevalence of HPV types 16 and 18 was observed to be 24.4%..

In our study the prevalence of co infection between HIV and Syphilis among the high risk group in our study was 14.2% which correlates with the study of Nimisha D. Shethwala et al[43] in which the prevalence of co infection was 14.28%.

The co infection of HIV and HSV2 among the high risk groups was 6(8.9%) which is more when compared with Yin YP et al[46] in which the prevalence of coinfection is 3.2% whereas in the study by Nilanjan Chakraborty et al[47] the prevalence of co infection between HSV2 and HIV is 47%

The co infection between HIV and HPV in our study was 25% which correlates with the study by Oliver et al[48] in which the prevalence of high risk human papilloma virus infection among the HIV positive individuals was 24.5%.According to Karen et al[52] bivalentHPV vaccine has been shown to protect against anal cancers caused by HPV types 16 and 18, which could reduce the prevalence of anal cancer in both men and women. Vaccination to be considered for high-risk groups that include FSW and MSM in order to implement a successful prevention of spread of disease[52].

The prevalence of co infection between HIV and candidiasis in our study was 5.5% which is low when compared with Nimisha D. Shethwala et al[43] in which the prevalence is 17.14%. One HIV reactive female sex worker is having both HPV 16 and HPV 18 infection which correlates with the fact that Human Immunodeficiency virus (HIV) positive women have significantly higher prevalence of genital squamous intraepithelial lesions and of multifocal HPV related diseases[29]

SUMMARY

This prospective study was conducted to find out the prevalenceof sexually transmitted infections with special preference to genital herpes infection and other microbial infections in the high risk groups attending STD clinic in a tertiary care hospital. Total of 200asymptomatic patients with 114 female sex workers 60 males having sex with males and 26 transgenders who attended STD clinic in Tertiary care Hospital between September 2013 to September 2014 were included.

Among the high risk groups taken for study the female sex workers (57%) were the commonest and most of them are unmarried 106(53%) with predominant mode of sexual contact was heterosexual mode (57%) and 41% had unprotected sexual act (without condoms).Specimens like Endocervical, High vaginal discharge along with urethral, rectal and pharyngeal swabs and blood were collected. Even with repeated motivation among the high risk group the willingness for Specimens like rectal swabs was low because of which disease prevalence was not fully known. These specimens were processed according to standard laboratory procedures to detect etiological pathogens.

Out of 200asymptomatic cases *Neisseria gonorrhoeae (1)*, *Trichomonas vaginalis (1)*, *Candidaspecies (18)*, *Gardernerella vaginalis (4)*were isolated. IgM IgM antibodiesfor *Herpes simplexvirus 2* is (16) and IgG(67). Eight with

HIV reactivity, 1 with HbsAg and seven with RPR reactivity for Syphilis were present in the high risk groups.

There was 100% agreement between direct gram staining and culture methods to detect *Neisseria gonorrhoeae* and the isolate was found to be resistant to Ciprofloxacin, Penicillin and Azithromycin and sensitive to Spectinomycin according to CLSI Disk diffusion method.

There was 100% agreement between direct gram staining and culture methods to detect *Trichomonas vaginalis*.

In candidiasis there was 100% agreement between direct gram stain and culture methods.Speciation of the candida species showed candida albicans 50% and non albicans 50%.

In Bacterial vaginosis the agreement between Nugent scoring, culture and the direct gram staining was66.6%.

The PCR analysis of the 200 anogenital swabs showed presence of Herpes *simplex virus 2* DNA glycoprotein gG 20.Out of 20 PCR positives IgG antibody was positive in all whereas the IgM was positive in only 16 by which it was evident that there is genital viral shedding even in the absence of IgM antibodies. Thus the interpretation of serological antibodies results reveals only the tip of the iceberg so to know about the genital viral shedding in the patients the use of molecular methods like PCR is mandatory.

The PCR analysis of the 200 anogenital swabs showed the presence of Human *Papilloma Virus 16 E7&E718* in 4 with one positive for both HPV 16 and 18 coinfection.

Among the 200 patients included in our study nine co infections exists (*Herpes simplex virus 2*with HIV in six, syphilis with HIV in one, HIV with Human *Papilloma Virus 16& 18* bothin one and Candida krusei with HIV in one).

CONCLUSION

In this prospective study among the high risk groups the female sex workers (57%) were the commonest and most of them are unmarried 106(53%) with predominant heterosexual mode of contact (57%) and 41% had unprotected sexual act .The prevalence of sexually transmitted infections in the 200 asymptomatic high risk groups was one rectal *Neisseria gonorrhoeae*, one *Trichomonas vaginalis*, eighteen *Candida species*, four *Gardernerella vaginalis* were isolated. Antibodies detected for *Herpes simplex virus 2 is* IgG in sixty seven and IgM in sixteen cases. Eight with HIV reactivity, one positive for HbsAg and seven with RPR reactivity for Syphilis were present in the high risk groups.

Out of 20 PCR positives the IgM was positive in only 16.There was genital viral shedding even in the absence of IgM antibodies. So molecular methods are necessary to know about the genital viral shedding.

The high risk *Human Papilloma Virus 16 & 18* was seen in 4 among the high risk groups.

This study showed unprotected sexual contact were high, so continuous efforts are needed to encourage individuals to adopt safer sexual practices by using condoms and to modify sexual behavior may substantially decrease the chance of STD transmission. Awareness among the FSW and MSM have to be emphasized on the significance of sexually transmitted infections.

Among the high risk groups the transgenders enrolled in the study were very low. Studies exclusively involving the transgenders will yield much data about the disease prevalence among this group.

Since the high risk group is a bridging population constant efforts are required to encourage the high risk group to adhere safer sexual practices and prophylactic strategies regarding antiviral therapy for HSV2 infected individuals and vaccination for HPV to be considered in the high risk groups.

ANNEXURES

APPENDIX

1) CHOCOLATE AGAR

Ingredients:

Nutrient agar base 30 gm.

Distilled water 1000 ml.

Sheep blood 100 ml.

Procedure:

1) Dissolve 30 g of Columbia agar base to 1 litre of distilled water in a flask.

Heat in a stem sterilizer to dissolve it completely.

2) Adjust the pH to 7.5 – 7.6.

3) Sterilize by autoclaving at 121° C for 15 minutes.

4) Cool to 70° C in a water bath.

5) Aseptically add 100 ml of sheep blood to it and leave at 70° C for 30 minutes.

6) Mix the blood and agar by gentle agitation from time to time till blood becomes chocolates brown in colour. This takes about 10 minutes.

7) Pour as slopes or plates in sterile tubes or sterile Petri dishes.

2) MODIFIED THAYER MARTIN MEDIUM WITH VCNT INHIBITORS

A selective medium for the growth of *N. gonorrhoeae*.

Ingredients:

1) MTM agar base 21 gm

2) 2% Hemoglobin solution 250 ml

3) Distilled water 450 ml.

4) VCNT inhibitor 10ml

i) Vancomycin 2.0 to 4.0 mg or Lincomycin 1 mg/litre

ii) Colistin 300.000 units to 7.5 mg/litre

iii) Nystain 12.5 IU/ml

iv)Trimethoprim 2mg/litre.

Procedure:

1) Mix 21 gms MTM agar in 450 ml of distilled water by boiling.

2) Sterilize by autoclaving at 121° C for 15 minutes.

3) Cool to a temperature of 50-55° C in a waterbath.

4) Aseptically add freshly prepared 250 ml of 2% hemoglobin solutionand

VCNT nhibitor to and mix well.

5) Pour 20 ml of medium in a sterile Petri dish of 90 mm diameterunder

strict aseptic precautions.

6) Allow the medium to cool and store in refrigerator.

3) PHOSPHATE BUFFERED SALINE:

Ingredients:

NaCl 9.0 gm

Potassium chloride 0.2 gm

Disodium hydrogen phosphate 1.15 gm

Potassium dihydrogen phosphate 0.2 gm

Distilled water 1000 ml

Mix the salts in distilled water. It gives a final pH 7.3.

4) SABOURAUD'S DEXTROSE AGAR

Ingredients:

Dextrose 40.0 gm

Neopeptone 10.0 gm

Agar 15.0 gm

Distilled water 1000 ml

Procedure:

1) Mix the ingredients in distilled water by boiling. Adjust pH to 5.6.

2) Sterilize by autoclaving at 115° C for 15 min.

3) Allow to cool to 50° C.

4) Add Chloramphenicol 1 mg/ml of medium under aseptic precautions.

5) Pour 20 ml amounts in 90mm Petri dish or into 15 ml test tubes.

6) Allow the test tubes to rest at an angle so that agar slopes (slants) are obtained.

7) After the medium solidifies, keep the Petri dish and cotton woolstopped

dextrose agar slants in the refrigerator.

5) DIAMOND'S TRICHOMONAS MEDIUM

Ingredients:

Trypticase 20.0 gm

Yeast extract 10.0 gm

Maltose 5.0 gm

L-Cysteine hydrochloride 1.0 gm

L-Ascorbic acid 0.2 gm

Di-potassium hydrogen phosphate 0.8 gm

Potassium dihydrogen phosphate 0.5 gm

Agar 0.5 gm

Distilled water 900 ml

Procedure:

1) Mix the contents in distilled water.

2) Autoclave the medium at 115°C for 20 minutes.

3) Cool the medium to 50° C and add 100 ml sheep or bovine serum (Sterilized by filtration) and 10 mgs % Chloramphenicol.

4) Store at 4° C till use.

6) Nutrient Agar

Ingredients Grams/litre

Peptic digest of animal tissue 5

Sodium Chloride 5

Beef extract 1.5

Yeast extract 1.5

Agar 15.00

Final pH(at 25º C) 7.4 ±0.2.

Suspend 28 gms in 1000 ml of distilled water. Heat toboiling to dissolve the medium completely. Sterilize byautoclaving at 15 lbs pressure (121ºC) for 15 minutes andpour into sterile petridishes.

7.) HBT Bilayer Medium Blood agar with Tween 80

Pancreatic Digest of Casein12.0 g

Peptic Digest of Animal Tissue 5.0 g

Yeast Extract 3.0 g

Beef Extract3.0 g

Peptone 10.0 g

Corn Starch1.0 g

Sodium Chloride5.0 g

Agar13.5 g

Polysorbate 80 0.075 g

Colistin10.0 mg

Nalidixic Acid20.0 mg

Amphotericin B3.0 mg

Human Blood, Anti coagulated (top layer only) 10%

PROCEDURE

HB(humanbloodbilayer)medium was composed ofabasallayerof7mlof CNA

Agarbase,withamphotericinB(2p.g/ml)Addedafterautoclavingwasperformed anda14-mlover layerofthesamecomposition plus 5% humanblood.1%Proteose PeptoneNo.3 was addedtobothlayersbeforeautoclavingwasperformed,and .0075%Tween80 was addedtobothlayersafterautoclavingwas performed.

8) CHROMagar Candida

REAGENTS

Chromopeptone10.0 g

Glucose 20.0 g

Chromogen Mix2.0 g

Chloramphenicol.0.5 g

Agar15.0 g

Suspend 47.5gms in 1000 ml of distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121ºC) for 15 minutes and pour into sterile petridishes.

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Key to Master Chart

KEY TO MASTER CHART

FSW-female sex workers

MSM-Males who have sex with males

TG-Transgenders

ART-Antiretroviral therapy

SRS-Sex Reassignment Surgery

A/O-anal and Oral

LMC-last marital contact

PMC-premarital contact

Prot-protected intercourse with condom

Unprot-unprotected intercourse

SURG-Surgery

BLD TRANS-Blood transfusion

END CX-Endocervical

H.VAGN-High vaginal

KOH-Potassium hydroxide

BYC-budding yeast cell

Intra cell GNDC-intra cellular gram negative diplococcic

GC cul-gonococcus culture

BV-Bacterial vaginosis

CPLM-Cysteine, peptic digest, Liver digest and Maltose

SDA-Sabourads dextrose agar

TV-Trichomonas vaginalis

HIV-Human Immuno Deficiency Virus

RPR-Rapid Plasma Reagin

TPHA-Treponema pallidum haemaggluttinin

HbsAg-Hepatitis B surface antigen

HCV-Hepatitis C virus

HSV IgM-Herpes simplex virus 2 IgM ELISA

HSV IgG-Herpes simplex virus 2 IgG ELISA

HPV-Human Papilloma virus

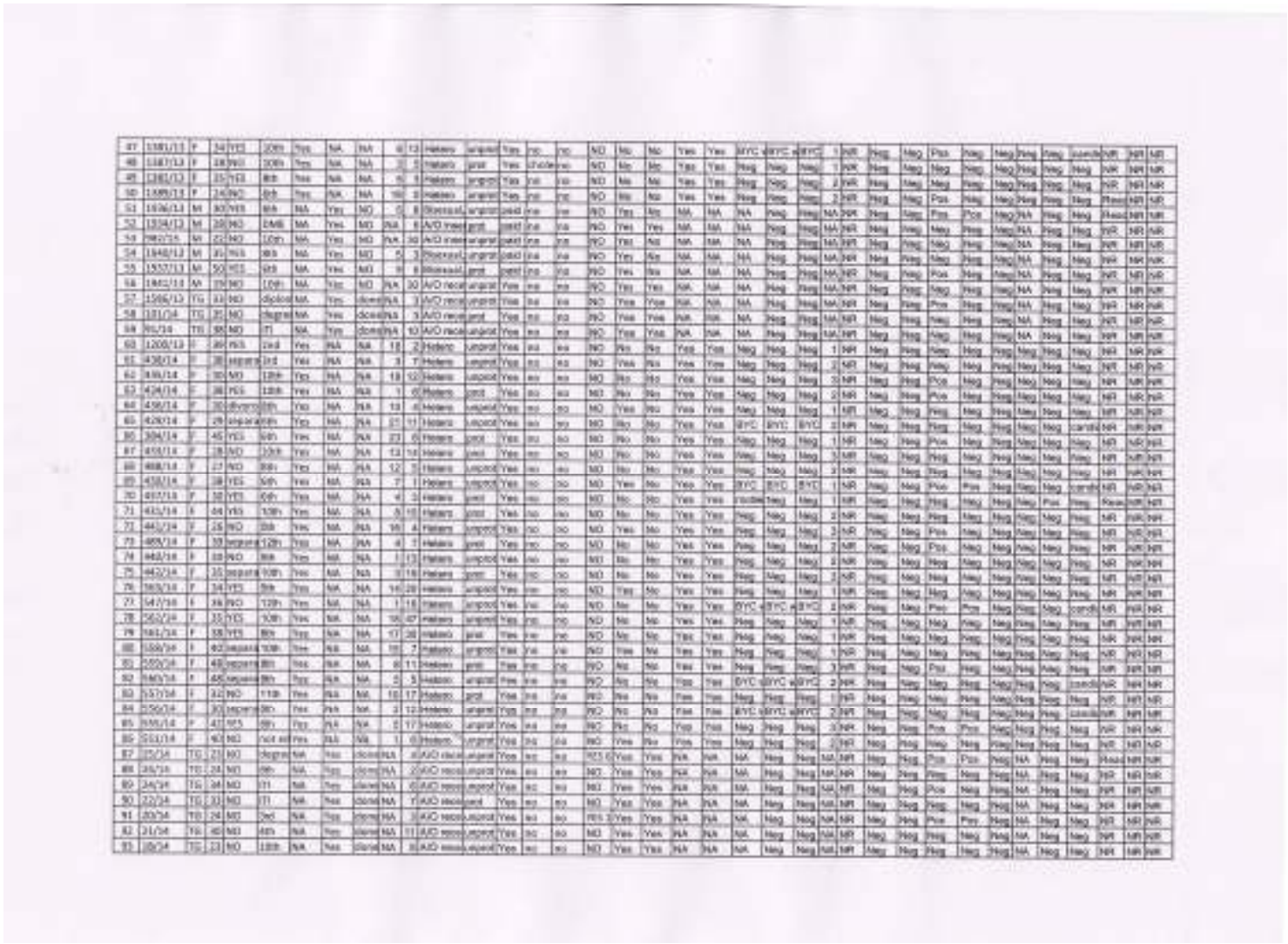
PCR-Polymerase Chain reaction

NR – Non reactive

Neg – Negative

Pos – Positive

SL.NO	STD NO.	SEX	AGE	MARIT	EDUCA	CSW	MSM	SRS	CLIMC	EM	ROUTE	PROTE	recei	H/O S	H/O B	PT OF	Phary	RECTA	END C	H.VAG	Wet m	KOH m	Gram	Nug	RPR/T	HBsAg	HCV	IgG HS	IgM H	Gono	Bacte	CPLM	SDA/D	HSV-2	HPV	HPV18
1	1199/13	F	27	YES	6th	Yes	NA	NA	10	5	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Clue c	Neg	Clue	8	NR	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Reac	NR	NR
2	1190/13	F	26	NO	12th	Yes	NA	NA	8	8	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
3	1206/13	F	39	separa	4th	Yes	NA	NA	4	6	Hetero	unprot	Yes	no	no	NO	No	No	Yes	Yes	BYC	BYC	BYC	1	NR	Neg	Neg	Pos	Neg	Neg	Neg	candi	NR	Rea	NR
4	1188/13	F	30	YES	8th	Yes	NA	NA	15	12	Hetero	unprot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
5	1210/13	F	31	separa	6th	Yes	NA	NA	20	5	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	0	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Reac	NR	NR
6	1195/13	F	36	YES	3rd	Yes	NA	NA	12	2	Hetero	prot	Yes	appes	no	NO	Yes	No	Yes	Yes	BYC	BYC	BYC	3	NR	Neg	Neg	Neg	Neg	Neg	Neg	candi	NR	NR	Reac
7	1185/13	F	30	NO	5th	Yes	NA	NA	4	12	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
8	1191/13	F	30	YES	8th	Yes	NA	NA	3	7	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Clue c	Neg	Clue	3	NR	Neg	Neg	Pos	Pos	Neg	Neg	Neg	Neg	Reac	NR	NR
9	1196/13	F	25	NO	3rd	Yes	NA	NA	2	3	Hetero	unprot	Yes	no	no	NO	Yes	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
10	1204/13	F	45	YES	not ad	Yes	NA	NA	1	5	Hetero	unprot	Yes	no	no	NO	No	No	Yes	Yes	Clue c	Neg	Clue	9	NR	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	NR	Rea	NR
11	1536/13	TG	24	NO	DEE	NA	Yes	2yrs	NA	3	A/O rece	unprot	Yes	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
12	1597/13	TG	24	NO	10th	NA	Yes	NO	NA	1	A/O rece	prot	Yes	tonsil	no	YES 2	Yes	Yes	NA	NA	Neg	Neg	Neg	NA	NR	Neg	Neg	Pos	Pos	Neg	NA	Neg	Neg	NR	NR	NR
13	1621/13	TG	22	NO	ITI	NA	Yes	2yrs	NA	10	A/O rece	unprot	Yes	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
14	1583/13	TG	30	NO	8th	NA	Yes	NO	NA	1	A/O rece	prot	Yes	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Pos	Neg	Neg	NA	Neg	Neg	NR	NR	NR
15	1593/13	TG	21	NO	BA	NA	Yes	2yrs	NA	3	Ano rece	unprot	Yes	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
16	1596/13	TG	41	NO	6th	NA	Yes	4yrs	NA	14	A/O rece	prot	Yes	no	no	YES 3	Yes	Yes	NA	NA	NA	Neg	Neg	NA	Reac	Neg	Neg	Pos	Pos	Neg	NA	Neg	Neg	NR	NR	NR
17	1592/13	TG	23	NO	8th	NA	Yes	NO	NA	3	A/O rece	prot	Yes	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
18	1624/13	TG	28	NO	diplon	NA	Yes	3yrs	NA	6	A/O rece	unprot	Yes	septo	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
19	1594/13	TG	26	NO	diplon	NA	Yes	2yrs	NA	8	A/O rece	unprot	Yes	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Pos	Neg	Neg	NA	Neg	Neg	Reac	NR	NR
20	1757/13	TG	30	NO	degre	NA	Yes	NO	NA	3	Ano rece	unprot	Yes	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Pos	NA	NR	Neg	Neg	Neg	Neg	Pos	NA	Neg	Neg	NR	NR	NR
21	1188/13	F	30	NO	10th	Yes	NA	NA	10	3	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
22	1208/13	F	40	NO	non ed	Yes	NA	NA	13	5	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	BYC	BYC	BYC	1	NR	Neg	Neg	Pos	Neg	Neg	Neg	candi	NR	NR	NR
23	1184/13	F	40	separa	not ed	Yes	NA	NA	4	2	Hetero	prot	Yes	no	no	yes 6	No	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Pos	Neg	Neg	Neg	Neg	NR	Rea	Reac
24	1192/13	F	21	separa	10th	Yes	NA	NA	6	3	Hetero	unprot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
25	1207/13	F	32	separa	8th	Yes	NA	NA	10	6	Hetero	prot	Yes	no	no	no	Yes	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
26	1266/13	F	35	YES	6th	Yes	NA	NA	15	7	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Reac	NR	NR
27	1263/13	F	32	YES	6th	Yes	NA	NA	18	8	Hetero	unprot	Yes	no	no	NO	No	No	Yes	Yes	BYC v	BYC v	BYC	1	NR	Neg	Neg	Neg	Neg	Neg	Neg	candi	NR	NR	NR
28	1759/13	F	25	NO	10th	Yes	NA	NA	3	11	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	3	NR	Neg	Neg	Pos	Neg	Neg	Neg	Neg	NR	NR	NR
29	1259/13	F	30	YES	8th	Yes	NA	NA	10	5	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Clue c	Neg	Clue	3	NR	Neg	Neg	Pos	Pos	Neg	Neg	Neg	Neg	NR	NR	NR
30	1386/13	F	31	NO	6th	Yes	NA	NA	22	5	Hetero	unprot	Yes	no	no	NO	Yes	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
31	1760/13	TG	22	NO	8th	NA	Yes	done	NA	3	Ano rece	unprot	Yes	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
32	1762/13	M	30	YES	12th	NA	Yes	NO	10	30	Bisexual	unprot	paid	no	no	NO	Yes	No	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Pos	Neg	Neg	NA	Neg	Neg	NR	NR	NR
33	1749/13	M	30	YES	6th	NA	Yes	NO	2	3	Bisexual	prot	paid	ORIF	Yes	NO	Yes	No	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	Reac	NR	NR
34	1212/13	M	26	NO	7th	NA	Yes	NO	NA	6	A/O inse	prot	paid	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
35	1748/13	M	31	YES	10th	NA	Yes	NO	2	6	Bisexual	unprot	paid	no	no	NO	Yes	No	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
36	1761/13	M	26	NO	4th	NA	Yes	NO	NA	9	A/O inse	unprot	paid	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Pos	Neg	Neg	NA	Neg	Neg	NR	NR	NR
37	1930/13	M	25	NO	12th	NA	Yes	NO	NA	3	Oro inse	prot	paid	no	no	NO	Yes	No	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
38	1938/13	M	26	NO	10th	NA	Yes	NO	NA	5	A/O inse	unprot	paid	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
39	1929/13	M	45	YES	10th	NA	Yes	NO	8	2	Bisexual	unprot	paid	no	no	NO	Yes	No	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Pos	Neg	Neg	NA	Neg	Neg	NR	NR	NR
40	1931/13	M	23	NO	Bsc	NA	Yes	NO	NA	1	A/O inse	prot	paid	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
41	1400/13	F	40	separa	3rd	Yes	NA	NA	4	7	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	BYC	BYC	BYC	2	NR	Neg	Neg	Pos	Pos	Neg	Neg	Neg	candi	Reac	NR	NR
42	1386/13	F	32	YES	10th	Yes	NA	NA	3	5	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
43	1385/13	F	33	YES	10th	Yes	NA	NA	13	9	Hetero	unprot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
44	1383/13	F	25	NO	8th	Yes	NA	NA	10	12	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	3	NR	Neg	Neg	Pos	Neg	Neg	Neg	Neg	NR	NR	NR
45	1398/13	F	33	YES	6th	Yes	NA	NA	5	6	Hetero	unprot	Yes	no	no	NO	Yes	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
46	1378/13	F	31	separa	6th	Yes	NA	NA	4	3	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Pos	Neg	Neg	Neg	Neg	NR	NR	NR



94	204/14	TG	30	NO	8th	NA	Yes	NO	NA	2	A/D rece	prot	Yes	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Pos	Neg	Neg	NA	Neg	Neg	Reac	NR	NR
95	891/14	M	45	YES	8th	NA	Yes	NO	2	4	Bisexual	prot	paid	no	no	YES 8	Yes	No	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Pos	Pos	Neg	NA	Neg	Neg	Reac	NR	NR
96	897/14	M	20	NO	6th	NA	Yes	NO	NA	1	A/D inse	unprot	paid	no	no	NO	Yes	No	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
97	895/14	M	46	YES	6th	NA	Yes	NO	1	3	Bisexual	unprot	paid	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
98	898/14	M	48	YES	10th	NA	Yes	NO	10	9	Bisexual	prot	paid	INSL S	no	NO	Yes	No	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Pos	Neg	Neg	NA	Neg	Neg	NR	NR	NR
99	32/14	F	23	NO	10th	Yes	NA	NA	4	5	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	5	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
100	33/14	TG	34	NO	8th	NA	Yes	DONE	10	3	A/D rece	unprot	Yes	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
101	35/14	TG	27	NO	6th	NA	Yes	NO	NA	9	A/D rece	prot	Yes	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Pos	Neg	Neg	NA	Neg	Neg	nonR	NR	NR
102	438/14	F	35	NO	5th	Yes	NA	NA	7	5	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
103	373/14	F	23	NO	8th	Yes	NA	NA	11	6	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
104	434/14	F	38	YES	3rd	Yes	NA	NA	1	3	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	BYC	3	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
105	428/14	F	27	NO	6th	Yes	NA	NA	19	2	Hetero	unprot	Yes	no	DONE	NO	No	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
106	431/14	F	44	separa	not ed	Yes	NA	NA	16	5	Hetero	unprot	Yes	no	no	YES 2	Yes	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Reac	NR	NR
107	1258/14	F	27	NO	6th	Yes	NA	NA	21	2	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
108	1259/13	F	38	YES	10th	Yes	NA	NA	15	7	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	BYC	BYC	BYC	2	NR	Neg	Neg	Pos	Pos	Neg	Neg	Neg	candi	Reac	NR	NR
109	1274/14	F	44	YES	10th	Yes	NA	NA	19	9	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	3	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
110	234/14	F	28	NO	8th	Yes	NA	NA	1	3	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
111	663/14	TG	22	NO	diplom	NA	Yes	NO	NA	4	A/D rece	prot	Yes	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
112	1089/14	F	29	NO	3rd	Yes	NA	NA	16	6	Hetero	prot	Yes	no	no	NO	Yes	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
113	1046/14	F	34	YES	10th	Yes	NA	NA	8	2	Hetero	unprot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
114	174/14	F	33	separa	5th	Yes	NA	NA	21	3	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	BYC	BYC	BYC	2	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	candi	NR	NR	NR
115	1052/14	F	43	YES	10th	Yes	NA	NA	3	1	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
116	1047/14	F	24	NO	8th	Yes	NA	NA	6	9	Hetero	prot	Yes	no	no	NO	Yes	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
117	1044/14	F	27	NO	6th	Yes	NA	NA	LW	1	Hetero	unprot	Yes	no	no	NO	No	No	Yes	Yes	BYC	BYC	BYC	2	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	candi	NR	NR	NR
118	983/14	F	30	NO	6th	Yes	NA	NA	10	5	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Pos	Pos	Neg	Neg	Neg	Neg	NR	NR	NR
119	838/14	F	36	YES	10th	Yes	NA	NA	10	4	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	3	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	candi	NR	NR	NR
120	861/14	F	26	NO	10th	Yes	no	NA	10	8	Hetero	prot	Yes	no	no	NO	Yes	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
121	826/14	F	25	NO	8th	Yes	NA	NA	2	4	Hetero	unprot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
122	854/14	F	32	separa	6th	Yes	NA	NA	6	3	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
123	802/14	F	42	YES	3rd	Yes	NA	NA	8	8	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
124	825/14	F	31	separa	10th	Yes	NA	NA	1	6	Hetero	prot	Yes	no	no	NO	Yes	No	Yes	Yes	Neg	Neg	Neg	6	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
125	834/14	F	38	YES	10th	Yes	NA	NA	6	2	Hetero	unprot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
126	451/14	F	25	NO	11th	Yes	NA	NA	9	4	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
127	663/14	F	22	NO	10th	Yes	NA	NA	7	3	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
128	823/14	F	38	YES	8th	Yes	NA	NA	3	6	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Clue	Neg	Clue	9	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg	NR	NR	NR
129	1085/13	F	30	YES	5th	Yes	NA	NA	1	3	Hetero	unprot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Reac	NR	NR
130	1210/13	M	32	YES	5th	NA	Yes	NO	8	5	Bisexual	prot	paid	no	no	NO	Yes	No	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
131	474/14	F	32	separa	10th	Yes	NA	NA	6	2	Hetero	prot	Yes	no	no	NO	Yes	No	Yes	Yes	Neg	Neg	Neg	1	Reac	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
132	537/14	F	26	NO	10th	Yes	NA	NA	11	8	Hetero	unprot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	2	Reac	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
133	797/14	F	22	NO	8th	Yes	NA	NA	15	5	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	2	Reac	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
134	1089/14	M	24	NO	6th	NA	Yes	NO	NA	7	A/D inse	unprot	paid	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	Reac	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
135	1666/14	M	28	NO	DCS	NA	Yes	NO	NA	6	A/D inse	prot	paid	no	no	NO	Yes	No	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Pos	Neg	Neg	NA	Neg	Neg	NR	NR	NR
136	1109/14	M	24	NO	10th	NA	Yes	NO	NA	3	A/D inse	prot	paid	no	no	NO	Yes	No	NA	NA	NA	Neg	Neg	NA	Reac	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	Reac	NR	NR
137	1122/14	F	42	YES	6th	Yes	NA	NA	17	8	Hetero	unprot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	1	NR	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
138	1126/14	F	30	YES	10th	Yes	NA	NA	10	7	Hetero	prot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	BYC	1	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
139	1128/14	F	35	YES	8th	Yes	NA	NA	1	5	Hetero	prot	Yes	no	no	NO	Yes	No	Yes	Yes	Neg	Neg	Neg	2	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR
140	1123/14	F	35	divorc	5th	Yes	NA	NA	12	8	Hetero	unprot	Yes	no	no	NO	No	No	Yes	Yes	Neg	Neg	Neg	3	NR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NR	NR	NR

188	2052/14	TG	33	NO	ITI	NA	Yes	NO	NA	3	A/D rece	prot	paid	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Pos	Neg	Neg	NA	Neg	Neg	NR	NR	NR
189	2055/14	M	24	NO	12th	NA	Yes	NO	NA	5	A/D inse	prot	paid	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
190	2057/14	M	25	NO	BCA	NA	Yes	NO	NA	7	Oro inse	prot	paid	no	no	YES 1	Yes	No	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Pos	Pos	Neg	NA	Neg	Neg	Reac	NR	NR
191	2059/14	M	29	NO	10th	NA	Yes	NO	NA	5	Bisexual	unprot	paid	toxicity	no	NO	Yes	No	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
192	2074/14	M	21	NO	4th	NA	Yes	NO	NA	8	A/D rece	prot	Yes	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
193	2075/14	M	32	NO	10th	NA	Yes	NO	NA	14	A/D inse	prot	paid	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
194	2078/14	M	34	NO	DME	NA	Yes	NO	NA	2	A/D inse	prot	paid	no	no	NO	Yes	No	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
195	2068/14	M	27	NO	BCA	NA	Yes	NO	NA	3	A/D inse	unprot	paid	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Pos	Neg	Neg	NA	Neg	Neg	NR	NR	NR
196	2069/14	M	28	NO	12th	NA	Yes	NO	NA	5	Ano inse	prot	paid	no	no	NO	Yes	No	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
197	2092/14	M	30	NO	8th	NA	Yes	NO	NA	7	A/D inse	prot	paid	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
198	2093/14	M	35	NO	10th	NA	Yes	NO	NA	6	A/D inse	prot	paid	no	no	NO	Yes	No	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
199	2118/14	M	24	NO	8th	NA	Yes	NO	NA	8	Oro inse	unprot	paid	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg	NR	NR	NR
200	2124/14	M	26	NO	10th	NA	Yes	NO	NA	2	A/D inse	prot	paid	no	no	NO	Yes	Yes	NA	NA	NA	Neg	Neg	NA	NR	Neg	Neg	Pos	Neg	Neg	NA	Neg	Neg	NR	NR	NR